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Volume LXXVII Number 1

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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AUGUST, 1939

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

FORTY-FIRST REPORT, FOR THE YEAR 1938—
FIFTY-FIRST YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W.

G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio,* who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members is fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and

define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1938.

The accounts have been audited by Messrs. Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1938, the book value of the Endowment Funds in the hands of the Central Hanover Bank and Trust Company as Trustee, was

General Fund,	Securities (market $862,409.23)	$ 916,855.70
	Real Estate	9,235.71
	Cash, principal	858.45
Library Fund,	Securities (market $162,008.68)	173,918.24
	Real Estate	20,102.88
	Cash	319.46
		$1,121,290.44

The income collected from these Funds was as follows:

General Endowment	$36,382.94
Library	6,665.66
	$43,048.60

The income in arrears on these Funds at the end of the year was:

Arrears *General Fund*	$13,518.86
Arrears *Library Fund*	3,450.00
	$16,968.86
Arrears at the end of the year 1937	$12,755.86
showing an increase of	$ 4,213.00

The dividends from the General Biological Supply House totalled $14,224.00.

Retirement Fund: A total of $4,060 was paid in pensions of which $197.20 was advanced from current funds. The Fund at the end of the year consisted of mortgages and real estate at the book value of $17,462.08.

Plant Assets: The land (exclusive of Gansett and Devil's Lane), buildings, equipment and library represent an investment of

	$1,789,884.74
less reserve for depreciation	517,178.00
or a net of	$1,272,706.74

The hurricane water damage to the inventory and equipment and plant amounted to $30,399.02 of which $2,387.97 was charged to Plant Fund and $28,011.05 to Current Surplus. Early this year The Carnegie Corporation of New York most generously contributed $20,000 toward the repair of the hurricane damage.

Income and Expenses: Income including a donation of stock valued at $7,250 exceeded expense, including $24,481.56 depreciation, by $11,432.64.

There was expended from current funds for plant account a net of $15,083.21 and in addition $6,500 in reduction of mortgage and note indebtedness.

At the end of the year the Laboratory owed $5,500 on mortgages and $7,000 on notes all for property purchased in earlier years. It had accounts and notes receivable of $12,305.69 and $8,531.29 in cash and bank accounts in its current funds.

A gift of 200 shares of Crane Company stock was received from Dr. Frank R. Lillie, to which he has since added 300 shares.

Following is the balance sheet, the condensed statement of income and outgo, and the surplus account all as set out by the accountants:

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1938

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee —Schedules I-a and I-b		$1,121,290.44	
Securities and Cash—Minor Funds—Schedule II		8,742.81	$1,130,033.25
Plant Assets:			
Land—Schedule IV	$ 110,884.58		
Buildings—Schedule IV	1,239,161.81		
Equipment—Schedule IV	165,567.34		
Library—Schedule IV	274,271.01	$1,789,884.74	
Less Reserve for Depreciation		517,178.00	
		$1,272,706.74	
Cash in Dormitory Building Fund		223.24	
Cash in Reserve Fund		24.65	$1,272,954.63
Current Assets:			
Cash		$ 8,531.29	
Accounts and Notes—Receivable		12,305.69	
Inventories:			
Supply Department	$ 37,672.27		
Biological Bulletin	9,762.64	47,434.91	
Investments:			
Devil's Lane Property	$ 44,398.34		
Gansett Property	5,822.49		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Stocks	7,250.00		
Securities and Real Estate—Retirement Fund List—Schedule V, viz.,			
Retirement Fund Portion	17,264.88		
Current Account Portion	197.20	87,632.91	
Prepaid Insurance		3,193.90	
Items in Suspense (Net)		693.98	$ 159,792.68
			$2,562,780.56

Liabilities

Endowment Funds:			
Endowment Funds—Schedule III	$1,120,581.61		
Reserve for Amortization of Bond Premiums	708.83	$1,121,290.44	
Minor Funds—Schedule III		8,742.81	$1,130,033.25
Plant Liabilities and Funds:			
Mortgage—Payable, Howes Property		$ 5,500.00	
Notes—Payable a/c Bar Neck Property Purchase .		7,000.00	
Donations and Gifts—Schedule III		1,038,402.61	
Other Investments in Plant from Gifts and Current Funds		222,052.02	$1,272,954.63
Current Liabilities and Surplus:			
Accounts—Payable		4,077.37	
Reserve for Additional Repairs and Replacements on account of Hurricane Water—Damage		17,518.12	
Current Surplus—Exhibit C.		138,197.19	159,792.68
			$2,562,780.56

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1938

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 36,382.94		$ 36,382.94
Library Fund		6,665.66		6,665.66
Donations		7,250.00		7,250.00
Instruction	8,356.14	9,960.00		1,603.86
Research	4,215.07	16,312.50		12,097.43
Evening Lectures	58.56		58.56	
Biological Bulletin and Membership Dues	9,691.33	10,362.75		671.42
Supply Department—Schedule VI .	40,814.01	38,134.93	2,679.08	
Mess—Schedule VII	25,899.67	25,759.83	139.84	
Dormitories—Schedule VIII	22,609.70	12,973.34	9,636.36	
(Interest and Depreciation charged to above 3 Departments —See Schedules VI, VII, and VIII)	*23,731.15*			23,731.15
Dividends, General Biological Supply House, Inc.		14,224.00		14,224.00
Rents:				
Bar Neck Property		3,568.46		3,568.46
Bay Shore Property	206.57	91.75	114.82	
Howes Property	196.64	480.00		283.36
Janitor House	23.19	360.00		336.81
Newman Cottage	81.43	250.00		168.57
Danchakoff Cottage	324.30	750.00		425.70

Sale of Library Duplicates		390.73		390.73
Apparatus Rental		991.30		991.30
Interest on Notes—Receivable		150.00		150.00
Sundry Income		38.54		38.54
Maintenance of Plant:				
Buildings and Grounds	22,482.48		22,482.48	
Chemical and Special Apparatus Expense	14,121.00		14,121.00	
Library Expense	7,576.77		7,576.77	
Truck Expense	1,249.70		1,249.70	
Workmen's Compensation Insurance	507.51		507.51	
Sundry Expense	19.50		19.50	
General Expenses:				
Administration Expense	12,199.94		12,199.94	
Endowment Fund Trustee and Safe-keeping	1,001.95		1,001.95	
Interest on Notes and Mortgage —Payable	829.83		829.83	
Bad Debts	448.39		448.39	
Reserve for Depreciation	24,481.56		24,481.56	
	$173,664.09	$185,096.73	$ 97,547.29	$108,979.93
Excess of income over Expense carried to Current Surplus—Exhibit C	11,432.64		11,432.64	
	$185,096.73		$108,979.93	

EXHIBIT C

Marine Biological Laboratory, Current Surplus Account, Year Ended December 31, 1938

Balance, January 1, 1938		$153,266.82
Add:		
Excess of Income over Expense for Year as shown in Exhibit B	$11,432.64	
Reserve for Depreciation Charged to Plant Funds	24,481.56	35,914.20
		$189,181.02
Deduct:		
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV,		
Buildings	$ 939.80	
Equipment	4,818.61	
Library	9,499.80	
	$15,258.21	
Less Received for Plant Assets Disposed of	175.00	
	$15,083.21	
Payment on Plant Mortgage and Note—Payable	$ 4,500.00	
Pensions Paid	$4,060.00	

Expenses on Account of Retirement Fund Securities	36.79		
	$4,096.79		
Less Retirement Fund Income and Gain from Security Sale	707.22	3,389.57	
Hurricane Water Damage (except portion Charged to Plant Funds)		28,011.05	50,983.83
Balance, December 31, 1938—Exhibit A			$138,197.19

Respectfully submitted,
LAWRASON RIGGS, JR.,
Treasurer.

V. THE REPORT OF THE LIBRARIAN

A report of the expenditures from the $18,800, appropriated to the Library in 1938, follows: books, $351.67; current serials, $5,319.86; binding, $1,171.38 ($45.00 of this on insurance); express, $181.48; supplies, $1,070.07 (includes $37.53 for new boxes to ship books to the bindery; $707.73 for new catalogue cases); salaries, $7,150.00; back sets, $1,795.33; total, $17,039.79.

For various reasons such as lack of space in the Library and the difficulty of securing the present lacks except in Germany, where prices are high, it seemed best to allow the $1,760.21 available for back sets besides $390.73 for the Library sale of duplicates, to revert to the General Fund of the Laboratory. Also a correction of the printed 1937 report must be made here. An order for the back set of "Flora" placed in Germany failed to come through and the order was finally cancelled by the Librarian, allowing another sum of $2,430.50 to drop from the Library expenditures.

The usual appropriation to the Library of $600.00 by the Woods Hole Oceanographic Institution was expended to the amount of $591.98 and separately accounted.

This year the Library lists but 1,306 current serials of which 426 are subscriptions, 385 (11 new) purchases of the Marine Biological Laboratory, 41 (1 new) of the Woods Hole Oceanographic Institution; 666 are exchanges, 596 (4 new) with the BIOLOGICAL BULLETIN and 70 (1 new) with the Woods Hole Oceanographic Institution publications; and 207 come as gifts to the former and 7 as gifts to the latter. The record shows 47 books purchased, 41 by the Marine Biological Laboratory and 6 by the Woods Hole Oceanographic Institution, 19 presented by the authors and 41 from publishers; while a contribution from Dr. Alfred Meyer enabled the Library to purchase a new "Ameri-

can Medical Directory"; and Dr. Douglas M. Whitaker presented a copy of Beaumont's "Experiments and Observations on the Gastric Juice and the Physiology of Digestion." Completed back sets of serials number 36; as purchases of the Marine Biological Laboratory, 20, of the Woods Hole Oceanographic Institution, 2; while purchases partially completing back sets number 15 for the former and 1 for the latter; through exchange of duplicates, 11 completed back sets for the former, and 1 for the latter; besides many additions to still incomplete back sets; and 2 sets for the Marine Biological Laboratory completed by gifts, with 4 partially completed. Reprint additions number 6,905: current for 1937, 1,897; current for 1938, 894, and of date previous to 1937, 4,114; about 200 of the latter kindly presented by Dr. M. A. Bigelow and 70 by Mrs. H. H. Donaldson. A summary of the current holdings of the Library proper is therefore 44,897 bound volumes and 108,927 reprints.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to present herewith a report of the fifty-first session of the Marine Biological Laboratory for the year 1938.

1. *Attendance.* The number of investigators and their assistants present during the summer of 1938 was somewhat less than in 1937, but it taxed the facilities of the Laboratory to the utmost. Attendance has risen steadily, with minor fluctuations, since 1933; now the number present is greater than the optimum which can be cared for under existing conditions. We are rapidly approaching the time when selection among the applicants for research space must be made, a situation referred to in the report of the Committee on Future Policy in the following words: "It will be necessary to adopt more definite policies concerning the admission of investigators than in the past. These should not, however, be of too binding a character, but rather a definition of principles within which the Director will have free scope for the exercise of his best judgment." The definition of these principles deserves the most careful consideration.

2. *The Library.* The continued growth of the Library is a source of satisfaction to the investigator, but it presents a serious problem to the Librarian who must find a place for new volumes and reprints. Each year's increment of bound volumes requires a space about equal to one complete stack. Since the present stacks are already practically filled, it will presently be necessary to use rooms now employed for cataloguing or other Library purposes. This will disturb the present orderly arrangement of serials and will at best provide only temporary relief. An addition to the Library is urgently needed.

3. *The Board of Trustees.* At the meeting of the Corporation held Tuesday, August 9, 1938, Dr. H. S. Jennings, Trustee since 1905, was elected Trustee Emeritus. To fill his place in the Class of 1942, Dr. M. H. Jacobs, the retiring Director, was chosen. At the same meeting, Dr. P. H. Armstrong was elected Clerk of the Corporation in place of Dr. Charles Packard who resigned when appointed Assistant Director.

The Board has suffered heavy losses by death. Mr. Charles R. Crane, Trustee from 1901 and President of the Board from 1902 to 1925, "the best friend the Laboratory ever had"; Dr. Edmund B. Wilson, Trustee continuously from 1890, whose contributions from this Laboratory were instrumental in establishing its scientific eminence; Dr. Charles R. Stockard, Trustee from 1920, whose counsels, vigorously expressed, were always highly valued; and Dr. J. Playfair McMurrich, Trustee from 1892 to 1900, active in the early days of this Laboratory.

4. *The Hurricane and Flood.* We may be profoundly thankful that in the storm of September 21, 1938 no one connected with the Laboratory lost his life. Some were rescued from desperate situations, and many suffered heavy material loss. Damage to Laboratory property was due almost entirely to water which poured into the basement of the Brick Building, into the Supply Department and the Dormitory, washed away most of the foundations of the Club House, and carried the Bathhouse far inland. The old laboratory buildings and the Mess were above the flood level.

During the height of the storm our staff worked heroically to protect the buildings and equipment. Mr. Larkin organized a bucket brigade and saved the apparatus in the Pump House; Mr. McInnis and his crew protected the motor boats; others barricaded doors in the Brick Building against the rising waters, but to no avail for the flood broke through the windows in the sub-basement of the Library; Mr. MacNaught opened the Apartment House to those who had been driven from their homes.

The greatest loss occurred in the Brick Building where the water, four feet deep, submerged microscopes and electrical apparatus, overran the storage battery, the switchboard and motors, and reduced the chemical and storage rooms to utter confusion.

The work of repair began at once. Dr. Pond and his assistants examined all the apparatus which had been wet with salt water, reconditioned much of it in our workship, and sent some to the manufacturers for servicing. To restore the Chemical Room required many weeks of hard work. Mr. McInnis and his men quickly reduced the confusion in the Supply Department where the damage was not great, and were

able, within a few days, to resume regular business. Under the direction of Mr. Larkin, the storage battery was cleaned and recharged, and the various motors were dried and set in place. None were lost, but some needed repairs. The switchboard was damaged but has been reconditioned. Mrs. Montgomery saved many of the more important duplicate reprints which had been water-soaked. Fortunately the regular reprint collection and the bound volumes were never in danger. The bathhouse, after being put back on new foundations, was damaged by a second storm. By order of the Executive Committee it was removed entirely.

These very extensive repairs to the buildings and the equipment have been made almost entirely by our permanent staff who have given unsparingly of their time and energy. To them the Laboratory owes a debt of gratitude.

In the Treasurer's Report the loss due to the storm is set at $30,400. This sum includes all of the various items which were lost. Inasmuch as many of these were of little actual value, and need not be replaced, the actual cost of restoring the damage will undoubtedly be less than $25,000. Since the Laboratory carried no insurance against this type of loss, the financial burden thus imposed upon us was serious. But we are fortunate in our friends. The Carnegie Corporation of New York, a benefactor of former years, has presented to the Laboratory the sum of $20,000 to be used for purposes of restoration. We are sincerely grateful for this generous and timely gift.

5. *Research in Botany.* For some years it has been apparent that the number of investigators at the Laboratory carrying on research in Botany has declined. This situation is due in part to the fact that some of the members of the Research Staff have been unable to attend the summer session, and in part to the lack of facilities for pursuing research in the dynamic phases of Botany. Following the resignation of Drs. Ivy M. Lewis, C. E. Allen and W. J. Robbins from the staff after many years of active service, Dr. E. W. Sinnott, of Columbia University, and Dr. D. R. Goddard, of the University of Rochester, were appointed. The lack of facilities for research has been stressed by many botanists who have expressed the opinion that more laboratory space is needed, that a suitable plot of ground for raising plants should be provided, and that a greenhouse is an essential part of an active botanical laboratory. These requirements should be met at the earliest opportunity.

6. *Gifts.* The sum of $20,000 given by the Carnegie Corporation of New York, to be used for the purpose of restoring the damage done by the flood, has already been mentioned. The Marine Biological Laboratory also gratefully acknowledges gifts amounting to $17,775 presented by Dr. F. R. Lillie.

7. *The Committee on Future Policy.* At the meeting of August 11, 1937, the Board of Trustees authorized the President to appoint a committee to formulate a statement concerning the policies and future of the Marine Biological Laboratory. The members of this Committee are: E. G. Conklin, Chairman, G. N. Calkins, W. C. Curtis, H. B. Goodrich, M. H. Jacobs, T. H. Morgan, G. H. Parker, A. C. Redfield and C. R. Stockard. After many discussions during the summers of 1937 and 1938 a report was drawn up by Dr. Lillie. This was studied and amended by the Committee and is now presented on p. 15 of this Annual Report.

8. *Lectures and Scientific Meetings.* During the summer of 1938 there were ten regular evening lectures and seven seminars at which shorter papers were discussed. In addition to these there were several informal exhibitions of motion pictures of scientific interest and a number of discussion groups. At the final scientific meetings, held August 30 and August 31, numerous investigators reported the results of their work during the current summer. In addition, many demonstrations were on display, both at the Laboratory and at the Fish Commission.

One of the regular seminar evenings was devoted to an informal celebration of the fiftieth anniversary of the founding of the Laboratory. Dr. Conklin reviewed the history of the early days, and Dr. Lillie spoke of those who have contributed to the scientific and material welfare of the institution. At the close of the meeting he presented to the Laboratory, in behalf of the Trustees, a portrait of Mr. Crane. It was a great source of satisfaction that Mr. Crane could be present to receive greetings from his many friends.

As in previous years, the Laboratory was host to the Genetics Society of America, which held its meetings on August 31 and September 1.

There are appended as parts of the report:

1. The Report of the Committee on Policies and Future of the Marine Biological Laboratory.
2. The Staff, 1938.
3. Investigators and Students, 1938.
4. A Tabular View of Attendance, 1934–38.
5. Subscribing and Coöperating Institutions, 1938.
6. Evening Lectures, 1938.
7. Shorter Scientific Papers, 1938.
8. General Scientific Meeting, 1938.
9. Members of the Corporation, 1938.

Respectfully submitted,

CHARLES PACKARD,
Associate Director.

1. REPORT OF THE COMMITTEE APPOINTED ON REQUEST OF THE BOARD OF TRUSTEES, AUGUST 10, 1937, TO FORMULATE A STATEMENT CONCERNING THE POLICIES AND FUTURE OF THE MARINE BIOLOGICAL LABORATORY

I. Introduction

By way of introduction, it is important to remind ourselves of the aims of the founders of the Marine Biological Laboratory. For this purpose a series of quotations follows. It is not the intention to present a history in any detail because it will be found that the original statements of policies and aims have been carefully observed during the entire history of the Laboratory for the fifty years of its existence. As the first director early remarked, "These policies should be the germ of an indefinite future development"; and this has been the case.

In the First Annual Report of the Marine Biological Laboratory for the year 1888, the Trustees made the following statements:

> "*Foundation.*—The Marine Biological Laboratory is an outgrowth of a sea-side laboratory maintained at Annisquam, Mass., from 1880 to 1886, by the Women's Education Association of Boston, in coöperation with the Boston Society of Natural History. In 1886, efforts were made by the Association to place the Laboratory on an independent and broader foundation. A circular letter was addressed to many of the leading biologists of the country, reciting what had been already done at Annisquam, and asking for coöperation and counsel. The replies received were most encouraging, testifying to a general and hearty approval of the enterprise, and promising coöperation and support." (P. 7.)

> "At the first meeting held by this committee, its members showed by votes that it was their desire to found a laboratory that should give opportunity for original research as well as for instruction, and soon after appointed the following

> Trustees

> Prof. William G. Farlow, Prof. Charles S. Minot,
> Miss Florence M. Cushing, Miss Susan Minns,
> Prof. Alpheus Hyatt, Prof. William T. Sedgwick,
> Mr. Samuel Wells." (P. 8.)

The first announcement issued in 1888 contained the following statements:

> "The Trustees of the Marine Biological Laboratory earnestly desire to enlist your co-operation in the support of a sea-side laboratory for instruction and investigation in Biology."

> "It is the desire of the Trustees that the enterprise shall enlist the active support of the universities and colleges of the country. To pre-

vent its becoming a simply local undertaking, they wish to see all who aid in its support by subscribing to investigators' tables share with the other members of the Corporation in the annual election of Trustees. The Trustees will, therefore, invite each institution which holds an investigator's table to name five persons for members of the Corporation during the term of subscription."

Dr. Whitman commented on these statements in the Eighth Annual Report, for the year 1895 as follows:

"Here we see sketched the elemental basis of our germ-organization —mainly potentialities of a theoretical nature, but 'instinct with spirit.' The aim was a permanent biological station; the function was to be instruction and investigation; the formative principle relied upon was co-operation." (P. 19.)

Whitman himself was the most influential person in determining the policies and aims of the new laboratory. In his first annual report as Director in 1888 he stated his personal viewpoint as follows:

"The new Laboratory at Woods Hole is nothing more, and, I trust, nothing less, than a first step towards the establishment of an ideal biological station, organized on a basis broad enough to represent all important features of the several types of laboratories hitherto known in Europe and America. It should be provided eventually with means for sending men to different points of the coast to undertake the investigation of subjects of special interest, thus adding to the advantages of a fixed station those of an itinerant laboratory.

"The research department should furnish just the elements required for the organization of a thoroughly efficient department of instruction. Other things being equal, the investigator is always the best instructor. The highest grade of instruction in any science can only be furnished by one who is thoroughly imbued with the scientific spirit, and who is actually engaged in original work. Hence the propriety—and, I may say, the necessity—of linking the function of instruction with that of investigation. The advantages of so doing are not by any means confined to one side. Teaching is beneficial to the investigator, and the highest powers of acquisition are never reached where the faculty of imparting is neglected. Teaching is an art twice blest; it blesseth him that gives and him that takes. To limit the work of the Laboratory to teaching would be a most serious mistake; and to exclude teaching would shut out the possibilities of the highest development. The combination of the two functions in mutually stimulating relations is a feature of the Laboratory to be strongly commended." (Pp. 16–17.)

In his lecture on "Specialization and Organization" (Biological Lectures, 1890) he remarked:

"Among the ways of bringing together our scattered forces into something like organic union, the most important, and the most urgent at

this moment, is that of a national marine biological station. Such an establishment, with a strong endowment, is unquestionably the great desideratum of American biology. There is no other means that would bring together so large a number of the leading naturalists of the country, and at the same time place them in such intimate helpful relations to one another. The larger the number of specialists working together, the more completely is the organized whole represented, and the greater and the more numerous the mutual advantages." (P. 24.)

In 1893 he wrote in his lecture on "Work and Aims of the Marine Biological Laboratory" (Biological Lectures, 1893):

"To those who by word and example have encouraged coöperation, this record will certainly be gratifying; and perhaps it will be accepted by all as an assurance that good-will and united effort have not been fruitless. For six years the Marine Biological Laboratory has stood for the first and the only coöperative organization in the interest of Marine Biology in America." (P. 236.)

The same year he remarked in his article "A Marine Observatory the Prime Need of American Biologists" (Atlantic Monthly, June, 1893, pp. 808–815):

"The Marine Biological Laboratory attaches itself to no single institution, but holds itself rigidly to the impartial function of serving all on the same terms. It depends not upon one faculty for its staff of instructors, but seeks the best men it can find among the higher institutions of the land. The board of trustees is a growing body, every year adding to its number, until it now comprises a very large proportion of the leading biologists of America. The whole policy is national in spirit and scope. The laboratory exists in the interest of biology at large, and not to nurse the prestige of any university or the pride of individual pretension." (P. 811.)

"Representative character, devotion to biology at large, independent government,—such are the essential elements of a strong and progressive organization." (P. 812.)

Again in 1898 he returned to the theme in an article "Some of the Functions and Features of a Biological Station" (*Science*, N.S., Vol. 7, No. 159, January 14, 1898, pp. 11–12):

"It now remains to briefly sketch the general character and to emphasize some of the leading features to be represented in a biological station.

"The first requisite is capacity for growth in all directions consistent with the symmetrical development of biology as a whole. The second requisite is the union of the two functions, research and instruction, in such relations as will best hold the work and the workers in the natural coördination essential to scientific progress and to individual development. It is on this basis that I would construct the ideal and test every practical issue.

"A scheme that excludes all limitations except such as nature prescribes is just broad enough to take in the science, and that does not strike me as at all extravagant or even as exceeding by a hair's breadth the essentials. Whoever feels it an advantage to be fettered by self-imposed limitations will part company with us here. If any one is troubled with the question: Of what use is an ideal too large to be realized? I will answer at once. It is the merit of this ideal that it can be realized just as every sound ideal can be realized, only by gradual growth. An ideal that could be realized all at once would exclude growth and leave nothing to be done but to work on in grooves. That is precisely the danger we are seeking to avoid.

"The two fundamental requisites which I have just defined scarcely need any amplification. Their implications, however, are far-reaching, and I may, therefore, point out a little more explicitly what is involved. I have made use of the term 'biological station' in preference to those in more common use, for the reason that my ideal rejects every artificial limitation that might check growth or force a one-sided development. I have in mind, then, not a station devoted exclusively to zoology, or exclusively to botany, or exclusively to physiology; not a station limited to the study of marine plants and animals; not a lacustral station dealing only with land and fresh-water faunas and floras; not a station limited to experimental work, but a genuine biological station, embracing all these important divisions, absolutely free of every artificial restriction.

"Now, that is a scheme than can grow just as fast as biology grows, and I am of the opinion that nothing short of it could ever adequately represent a national center of instruction and research in biology. Vast as the scheme is, at least in its possibilities, it is a true germ, all the principal parts of which could be realized in respectable beginnings in a very few years and at no enormous expense. With scarcely anything beyond our hands to work with, we have already succeeded in getting zoology and botany well started at Woods Hole, and physiology is ready to follow."

II. Future Plans and Policies

A. The Problem of Expansion vs. Consolidation

Since the erection of the "New Laboratory" in 1923, there has been a steady growth in the attendance of investigators, subject to some recession during the depression, but reaching a peak in 1937 which strained our accommodations to the limit during the greater part of the session. The question is therefore forced upon our attention whether we should limit arbitrarily the number of investigators as we have long since done in the case of students in classes. The only alternative would be to increase our accommodations. Decision of this point would affect various policies, and it should therefore receive first consideration.

The Committee have given careful attention to the question of expansion and have reached the unanimous conclusion that it would be wise at this time to consolidate and develop our present plant and organization, and to postpone the question of expansion, or of new construction except as noted below under Library and under Instruction.

The main reasons for this opinion are two: first, that the problems of housing and adequate care of a considerably larger number of persons would be difficult in the restricted community in which we find ourselves, and second, the need of prudence which rests upon economic uncertainties. It is by no means certain that we may not have to face another period of depression before many years, and this should not find us over-expanded. Each of these considerations can, of course, be developed in detail.

B. The Principle of Coöperation

Whitman spoke of coöperation as the "formative principle" of the Laboratory. It is illustrated in the national scope of the Laboratory and in its fundamental organization and government. The principles involved in nation-wide institutional representation and coöperation, and in comprehensive membership of the Corporation, are so rooted in our practices and have proved so fruitful as to require only emphasis.

C. Organization and Government

The inter-relations of Trustees and Corporation as given in the By-laws have operated harmoniously and effectively for a long time.

Rules concerning nomination and election of Trustees and members of the Corporation by the respective bodies have been formulated as follows:

1. By the Corporation:—August 11, 1931.

1) After considering various methods by which those engaged in instruction might be represented upon the Board of Trustees, it is believed that the following action by the Corporation will be the best means of insuring such representation:

"The Corporation affirms its position that instruction in course work is a fundamental part of the work of the Laboratory and should be adequately represented upon the Board of Trustees."

2) "That the Committee of the Corporation for nomination of Trustees consist of five members, of whom not less than two shall be non-Trustee members and not less than two shall be Trustee members of the Corporation."

3) "That on or about July first of each year, the Clerk shall send a circular letter to each member of the Corporation giving the names of the Nominating Committee and stating that this committee desires suggestions regarding nomination."

4) "That the Nominating Committee shall post the list of nominations at least one week in advance of the annual meeting of the Corporation."

(Memo: The same committee also makes nominations annually for Treasurer and Clerk of the Corporation.)

2. By the Trustees:—August 10, 1937.

"Proposals for membership in the Corporation shall be made to the Nominating Committee on or before the first Tuesday of August upon a regular form and endorsed by two members of the Corporation.

"With the recognition that rigid and completely standardized requirements for membership in the Corporation of the Marine Biological Laboratory are neither practicable nor desirable, it is recommended that future members of the Corporation shall, in general, be selected from among persons who, by engaging in active research at the Marine Biological Laboratory during substantial portions of at least two summers, shall have become acquainted with the work, aims, and peculiar problems of the Laboratory, and who, by papers published over a period of several years shall have demonstrated a capacity for sustained scientific productiveness not less than that required for full membership in such national societies as the American Society of Zoölogists, the Botanical Society of America, and the American Physiological Society.

"It is further recommended that in doubtful or border-line cases action on applications for membership shall be deferred until a time when, in the opinion of the Nominating Committee then serving, the status of the applicant has become entirely clear."

D. Administration

In the course of the years we have developed methods of administration of the various service departments of the Laboratory that have worked well. It should be the function of the Director and Assistant Director to control the operation of such services.

Dr. Jacobs' greatly regretted resignation as Director raises very directly the question of the higher administration. The first two Directors of the Laboratory served without salary, and the routine admin-

istration was performed by an Assistant Director on pay, at first part time but later on full time. Then Dr. Jacobs performed the services both of Director and Assistant Director on half time and half pay, and the Business Manager became able with experience to take over many of the duties formerly exercised by the Assistant Director. Though this arrangement worked admirably for the period of its duration, experience showed that it is not reasonable to expect a man of the scientific experience and reputation expected of the Director of this Laboratory to endure indefinitely the limitations of scientific activity imposed by such an arrangement. It seems probable that we cannot return to this plan.

As soon as possible we should provide for a full-time resident Director or Assistant Director. This would afford continuous supervision of the business of the Laboratory and in addition would permit this officer to continue his research work under favorable conditions. Such a resident scientist would attract other scientists during the portion of the year when the Laboratory is little used and would thus help to make it an all-year-round institution.

E. Research and Instruction

Research and instruction have been companion principles since the foundation of the Laboratory as cited in the introduction to this report. In the maintenance of research and instruction side by side throughout its history, the Marine Biological Laboratory has been outstanding, if not strictly unique. We have stood by the principle that it is the business of the Laboratory to help to produce investigators as well as investigation; and we believe that it can be shown that our courses of instruction have contributed in an important way to this purpose, and, moreover, that they have been an important factor in the improvement of biological instruction and research throughout the country. Although there has been some opinion among members of the Laboratory since the courses ceased to be an important source of income that we would be better off without courses, this opinion has never prevailed. We believe that our problem is in the way of improvement, not elimination, of instruction.

The Laboratory has no program of its own in research, except as defined in its name, and it therefore promotes no specific research projects as official undertakings. It operates entirely on the principle of furnishing facilities to competent investigators, and to beginning investigators who are working under qualified direction. No biological subjects are specifically excluded except such as are ruled out by lack of facilities or suitable conditions, as in the case of pathogenic organisms for example. This has been the rule from the foundation of the Labora-

tory, and the range of research has consequently steadily increased with improvement of facilities. Changes of fashion have of course also occurred, and are reflected in the annual reports.

The policy has been to interest the strongest biologists and promising young investigators to bring their work to Woods Hole; and the degree of success of this policy has been the measure of success and influence of the Laboratory. The future of the Laboratory depends upon the continuance of this policy, and the elimination of conditions that tend to restrict its operation, whether these are based on inadequacy of equipment, administrative regulations, or community conditions. This is the most important policy of the Laboratory, if one may be allowed to rank essentials, for it ensures leadership and reputation. To supplement this policy the attendance of as many promising young investigators as possible should be encouraged.

If the number of investigators admitted is to be definitely restricted, and if the tendency towards an increase in numbers continues, it will be necessary to adopt more definite policies concerning admission of investigators than in the past. These should not, however, be of too binding a character, but rather a definition of principles within which the Director will have free scope for the exercise of his best judgment.

The established fees for research accommodations should be continued, and paid by the institution represented as far as possible. When this cannot be done it has been a frequent policy, more in the past than at present, to waive fees for distinguished investigators. Such arrangements have often been doubly blessed, in giving and in taking. The coöperation by institutions in the expenses of investigation of their representatives has been a strong stabilizing factor in the history of the Laboratory in more ways than one. This plan has never been more effective than at the present time, but it is important constantly to cultivate it.

The Committee recommends the continuance of our historical policy of maintaining courses of instruction. These should be contributory to research, and based upon the advantages of marine material, so that they are in no sense duplications of courses that may equally well be offered by universities. Of such courses there are several kinds. As contributory to research it is not meant that all necessarily lead directly to research as a final preparatory step, but that they may sometimes fill essential gaps in education for the kind of biological research intended by the individual. Preference for admission to courses should be given to students whose promise or declared intention indicates a professional career in the field of biology. Such students should, and do, derive great profit, not only from the actual instruction, but also from the scientific contacts that they make at Woods Hole.

The Trustees should maintain control of courses to see that proper content and principles of admission are preserved. The Executive Committee has for some time held a conference with the heads of courses each year with these purposes in mind.

Strict limitation of the numbers admitted to each course should be observed in the future as in the past. It should also be a policy to provide better and more stable laboratory accommodations.

F. Buildings, Equipmnt and Grounds

The first question is whether our holdings of real estate are adequate for the future. This can be answered substantially in the affirmative. We already have considerable undeveloped harbor frontage; we now own all the land on the block on which the original buildings of the Laboratory stood; in the block immediately north there is only one parcel of land on Center Street not now in our possession; and there is no immediate reason for attempting to complete our ownership of the remainder of the block. For residential purposes we still have unsold lots in the Gansett tract, and no subdivision whatever has been made of the 100 acres in the Devil's Lane Tract.

The second question concerns the buildings. Here three main needs present themselves.

In the first place, additional stack space for the library is needed. At the present rate of growth the stacks will be fully occupied in very few years. It is essential for the work of the Laboratory that this growth should be continued. Additional space can be provided by a wing to the east of the present library. It has been suggested that the present reading room might be utilized for additional stack space and the catalogue room be converted into a reading room with other necessary readjustments; other suggestions for temporary relief have also been offered. But at most only a short postponement would be afforded in such ways. The problem should be faced and estimates secured for building additional stack space.

The second main need is to replace the present wooden buildings with a fireproof building of solid construction. The work of the classes and investigators in the wooden buildings is seriously hampered by vibration, and the buildings do not lend themselves readily to modern installations. These buildings range in age from forty to fifty years, and they constitute a real fire hazard. This need should also receive the earnest attention of the Trustees.

Additional space is also needed for various technical services necessitated by the increasing complexity of important kinds of biological research in recent years, and which are not adequately provided for at

present. Among these needs are those for space for autoclaves and sterilizers, which must now be used in rooms occupied by investigators, space for stills, which are now very disadvantageously housed in the boiler room in the basement of the Brick Building, additional shop space, particularly for use by investigators for relatively simple operations which they can carry out themselves, additional space for housing small animals, dehumidified and air-conditioned rooms, additional dark rooms, etc. Doubtless most of these needs could be cared for on the lower floors of the proposed addition for the Library. They ought, in any event, not to be forgotten. Furthermore, since needs of this sort are likely to increase in future years and are less predictable than the growth of the library, ample reserve space should be provided for them.

Our waterfront should be improved by landscaping and other ways so as to furnish a dignified frontage and water approach to the Laboratory. The George M. Gray Museum should have more adequate housing, and there are numerous other desirable small improvements that should be undertaken as soon as possible.

It is becoming increasingly important that the Supply Department be enabled to collect material for research from a wider area. To this end there should be a larger motor boat, and it is highly desirable that a resident naturalist be associated with the department who could study ecological conditions from year to year with a view to establishing sources of more abundant and more varied material for research. The standing Committee in the Supply Department should be asked to formulate the aims and policies of the Department.

G. Library

The Library Committee should be asked to formulate the aims and policies of the library.

H. Apparatus

Similarly, the Apparatus Committee should be asked to formulate its aims and policies.

I. Finances and Fiscal Policies

In 1932 the income from our endowment funds was $55,668, representing a return of 5 per cent on book value. It is now approximately $43,000, representing a return of 3.8 per cent on book value. The decrease in yield has been due partly to the necessity of refunding operations at lower interest rates; but the most drastic reductions in income have been suffered on the mortgage participations, some of which have been foreclosed, and others have had the interest rate much reduced.

The outstanding arrears of income amounted to $18,094 in 1935 but were reduced to $12,775 at the end of 1937. For three years the income has been supported by payment of arrears, a condition that cannot continue indefinitely.

With a loss of annual income from endowment amounting to over $12,000 there has been a considerable increase in attendance, which has not been compensated for by increased fees for research space. The cost of most apparatus and materials has recently risen appreciably and is likely to rise still farther. It is also certain that the progress of biological research will continually create new demands for special apparatus and equipment which must be met if the Laboratory is to retain its present position in scientific research. As a matter of fact, the budget of the Laboratory has been kept balanced since the beginning of the depression only by economies which have considerably handicapped the work of many of our investigators. Furthermore, although necessary upkeep has been maintained, certain desirable repairs to the buildings and equipment have been postponed since the early years of the depression but cannot be deferred much longer. Among the more expensive items that will require attention within the next few years are battery replacement, a new heating system for the Brick Building, repairs of the salt water system, painting and waterproofing of the brick buildings, etc. Reserves should also be built up to cover further depreciation of the buildings and equipment owned by the Laboratory, and to provide for the retirement of the Howes mortgage and for future purchases of property, etc. The problem of sewage disposal which may arise at any time is also likely to involve very considerable expense.

It is clear that substantial increase of the endowment of the Laboratory is necessary if we are to aim to restore the income to its predepression value, to provide adequately for the upkeep of the present plant, for the establishment of necessary reserves, and to meet increasing costs of operation.

As a partial offset to the loss of endowment income since 1931, the dividends of the General Biological Supply House increased from $2,032 in 1931 to $12,700 in 1936. The income from fees of students and investigators cannot be increased much unless considerably higher rates are established, which seems undesirable.

The Committee agrees that the most important fiscal policies to pursue are first to increase endowment and second to establish cash reserves for depreciation and contingencies. It is fair to point out in the latter connection that cash reserves previously accumulated have been used for purchase of real estate and that considerable sums have also gone each year into capital improvements. The Committee recommends that

additional endowments secured be placed in the same trusts as the present major endowment funds, or in another trust under the same principles.

J. Community Arrangements and Responsibilities

As our community has grown and assumed a more settled character, community needs have increased. The primitive needs of food and lodging have from the beginning been recognized as an official responsibility of the Laboratory; the present arrangements for low-cost housing do not appear to be entirely adequate for a community of our size. Their administration should be in the hands of the Business Manager subject to control by the superior administrative officers. An advisory committee is not recommended.

For those who desire to own their own homes, the Laboratory possesses ample real estate in the Gansett and Devil's Lane tracts, saleable to members at reasonable rates and terms. The acquisition of these tracts has aided to prevent unreasonable increase of price of village properties.

These provisions should be regarded as terminating the direct and exclusive official responsibility of the Laboratory for community purposes. While the Laboratory should aid in securing recreational facilities, the responsibility for operating them should be in the hands of the community itself. This principle has operated well in the case of the "M. B. L. Club" and the "M. B. L. Tennis Club." The Laboratory has furnished land and buildings, and from time to time has made loans for improvements, and it may yet appear desirable to provide an addition to the building of the M. B. L. Club. But these organizations should operate under their own membership and fees. With the acquisition of the bathing beach the question arises whether the same principles could not be made to operate there.

K. Summary of Principal Recommendations

1. That the Marine Biological Laboratory pursue a policy of consolidation rather than expansion for the present.
2. That in pursuit of this policy steps be taken to provide the following major improvements:

   a. Secure additional funds for endowment.
   b. Provide additional stack room to accommodate approximately 100,000 additional volumes, together with study cubicles.
   c. Replace present wooden laboratories by a building, or buildings, of stable fireproof construction, providing an intermediate court to set off the present main building.

d. In connection with the library construction provide adequate space for expansion of various technical services as described in II F.

e. Make provision for the series of miscellaneous needs enumerated in the body of the report.

3. Maintain the principles of coöperation (II B.), organization and government (II C.), administration (II D.), research and instruction, (II E.), that have served so well in the past, as the basis for future development.
4. Additional endowment funds as received should be placed, like the present main endowment funds, in trust. Reserves for depreciation, contingencies, improvements and retirement fund should be set up out of income (II I.).
5. Responsibility for recreational facilities should be placed as far as possible on voluntary organizations within our scientific community (II J.).

2. THE STAFF, 1938

CHARLES PACKARD, Associate Director, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
E. G. CONKLIN, Professor of Zoölogy, Princeton University.
CASWELL GRAVE, Professor of Zoölogy, Washington University.
H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.
FRANK R. LILLIE, Professor of Embryology Emeritus, The University of Chicago.
C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.
S. O. MAST, Professor of Zoölogy, Johns Hopkins University.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoölogy Emeritus, Harvard University.
E. B. WILSON, Professor of Zoölogy Emeritus, Columbia University.
LORANDE L. WOODRUFF, Professor of Protozoölogy, Yale University.

II. INSTRUCTION

T. H. BISSONNETTE, Professor of Biology, Trinity College.
P. S. CROWELL, JR., Instructor in Zoölogy, Miami University.
C. E. HADLEY, Associate Professor of Biology, New Jersey State Teachers College at Montclair.
F. R. KILLE, Assistant Professor of Zoölogy, Swarthmore College.
A. M. LUCAS, Associate Professor of Zoölogy, Iowa State College.
S. A. MATTHEWS, Assistant Professor of Biology, Williams College.
A. J. WATERMAN, Assistant Professor of Biology, Williams College.

Junior Instructors

W. F. Hahnert, Associate Professor of Zoölogy, Ohio Wesleyan University.
J. S. Rankin, Teaching Fellow in Zoölogy, Amherst College.

PROTOZOÖLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
G. W. Kidder, Assistant Professor of Biology, Brown University.
Elizabeth Drumtra Hughes, Lecturer in Zoölogy, Barnard College.

EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

W. W. Ballard, Assistant Professor of Biology and Anatomy, Dartmouth College.
Hubert B. Goodrich, Professor of Biology, Wesleyan University.
Viktor Hamburger, Assistant Professor of Zoölogy, Washington University.
Oscar Schotté, Assistant Professor of Biology, Amherst College.
Douglas M. Whitaker, Professor of Zoölogy, Stanford University.

PHYSIOLOGY

I. Investigation

William R. Amberson, Professor of Physiology, University of Maryland, School of Medicine.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
M. H. Jacobs, Professor of General Physiology, University of Pennsylvania.
Ralph S. Lillie, Professor of General Physiology, The University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

Laurence Irving, Professor of Biology, Swarthmore College.
Robert Chambers, Professor of Biology, New York University.

J. K. W. Ferguson, Assistant Professor of Physiology, Ohio State University.
Kenneth C. Fisher, Assistant Professor of Experimental Biology, University of Toronto.
C. Ladd Prosser, Assistant Professor of Physiology, Clark University.
Carl F. Schmidt, Professor of Pharmacology, University of Pennsylvania.
F. J. M. Sichel, Instructor in Physiology, University of Vermont, College of Medicine.

BOTANY

I. Investigation

C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Michigan.
Francis Drouet, Research Fellow, Yale University.
B. F. D. Runk, Research Fellow, University of Virginia.

GENERAL OFFICE

F. M. MacNaught, Business Manager.
Polly L. Crowell, Assistant.
Edith Billings, Secretary.

RESEARCH SERVICE AND GENERAL MAINTENANCE

Samuel E. Pond, Technical Mgr.
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Students

BOTANY

Bader, Joan E., Montclair State Teachers College.
Bien, Bettina H., Student, Wheaton College.
Bonner, John T., Student, Harvard University.
Fender, Flora S., Preparator, University of Pennsylvania.
Graves, E. Irene, Senior Instructor in Biology, Bridgewater State Teachers College.
Hoffman, Elizabeth D., Mount Holyoke College.
Markle, Jane C., Smith College.
Postel, Frances H., Wellesley College.
Rutledge, Alma W., Graduate Student, Johns Hopkins University.
Schallek, William B., Harvard University.
Siegel, Marion T., New Jersey College for Women.
Ward, Henry S., Jr., Alabama Polytechnic Institute.

EMBRYOLOGY

Alley, Armine, Research Assistant, McGill University.
Armstrong, Florence H., Student, Dalhousie University.
Berry, Clyde, Jr., Washington University.
Blanchard, Joseph, Student, Wesleyan University.
Bookhout, Cazlyn G., Instructor in Zoölogy, Duke University.
Brush, Helen V., Vassar College.
Collier, Jane G., Assistant, University of Missouri.
Coppola, Armando R., Brothers College of Drew University.
Dorler, Marian, Goucher College.
Drury, Horace F., Harvard University.

DUNHAM, DONALD W., Assistant in Zoölogy, Ohio State University.
EDDS, MAC VINCENT, JR., Amherst College.
FINK, HAROLD K., Graduate Student, Princeton University.
FINKEL, ASHER J., Research Assistant, The University of Chicago.
HARROLD, CHARLES M., JR., Graduate Assistant, New York University.
KLEIN, ETHEL L., University of Rochester.
KURTZ, ELIZABETH L., Wilson College.
LEWISOHN, MARJORIE G., University of Michigan.
MILNE, WALTER S., Graduate Assistant, University of Missouri.
PHILIPS, FREDERICK S., Graduate Assistant, University of Rochester.
ROGICK, MARY D., Professor of Biology, College of New Rochelle.
ROGOFF, WILLIAM M., Graduate Student, Yale University.
ROTHERMEL, JULIA E., Professor of Biology, Western College.
SODERWALL, ARNOLD L., Assistant in Zoölogy, University of Illinois.
SPANGLER, JULIET M., Wheaton College.
STABLEFORD, LOUIS T., Laboratory Assistant, Yale University.
STEVENS, FLORENCE F., New Jersey College for Women.
TAYLOR, HARRIETT E., Radcliffe College.
TERZIAN, ANNETTE V., Mount Holyoke College.
TOWLE, HARRIET N., Assistant in Zoölogy, Wellesley College.
WADDILL, SAMUEL F., Washington and Jefferson College.
WILLIAMS, JOHN L., Graduate Assistant, New York University.
WOODWARD, ARTHUR A., JR., Student, Oberlin College.
WORDEN, FREDERIC G., Student, Dartmouth College.

PHYSIOLOGY

ALBRINK, WILHELM S., Assistant in Biology, Yale University.
ALLEN, PAUL J., Graduate Assistant in Botany, University of Rochester.
ARMSTRONG, CHARLES W. J., Demonstrator in Biology, University of Toronto.
BECK, NAOMI E., The University of Chicago.
BLAIR, JOHN H., Graduate Assistant, Wesleyan University.
BLISS, ALFRED F., Columbia University.
BRISCOE, PRISCILLA M., Graduate Student, Ohio State University.
CASEY, MARGARET T., Graduate Assistant in Physiology, Mount Holyoke College.
CROWELL, HAMBLIN H., Graduate Assistant, Ohio State University.
CURTIS, HOWARD J., Fellow, Rockefeller Foundation.
GRAVE, CASWELL, II, Assistant, Washington University.
HENSON, MARGARET, Assistant in Physiology, Wellesley College.
LEVINE, HARRY PHILIP, Zoölogy Instructor, University of Vermont.
MARTIN, ROSEMARY D. C., Assistant, University of Toronto.
MOORE, IMOGENE, Instructor in Zoölogy, New Jersey College for Women.
MULLINS, LORIN J., University of California.
O'BRIEN, JOHN P., Johns Hopkins University.
OWENS, WILLIAM C., St. John's College.
SMITH, AUDREY U., Assistant in Physiology, Vassar College.
VON DACH, HERMAN, Assistant in Zoölogy, Ohio State University.
WIEGHARD, CHARLOTTE, 4544 Harris Avenue, St. Louis, Missouri.
WILSON, JOHN W., Graduate Assistant in Zoölogy, Duke University.

PROTOZOÖLOGY

BEVEL, NELL H., Assistant in Zoölogy, Duke University.
BURBANCK, WILLIAM D., Graduate Assistant, The University of Chicago.
COLE, ROGER M., Undergraduate Assistant, Massachusetts State College.
EWING, WILLIAM H., Fellow in Biology, Washington and Jefferson College.

FINKELSTEIN, NATHANIEL, Johns Hopkins University.
HIERHOLZER, CAROLYN ANNE, Instructor in Biology, Adelphia College.
KORNBLUM, LUCILE, Student, Columbia University.
MAYO, MERCEDES, Assistant Professor of Biology, Universidad de la Habana.
WELLS, WAYNE W., Associate Professor of Science, Southern Oregon State Normal.
WILKINSON, ELIZABETH J., Student, Columbia University.

INVERTEBRATE ZOÖLOGY

ACOSTA, JOSEFINA, Goucher College.
ALEXANDER, ROBERT S., Graduate Assistant, Amherst College.
ARNSTEIN, MARGERY, Simmons College.
BELDA, WALTER H., Graduate Student, Johns Hopkins University.
BIGLER, FRANCES B., Western Reserve University.
BROWN, HENRY, Student, College of the City of New York.
COONEY, MARILYN R., Student, Smith College.
CRANE, TODD, Student, Wilson College.
DAVIS, JAMES O., Graduate Assistant in Zoölogy, University of Missouri.
DELISA, DOMINICK A., Student, Union College.
DERINGER, MARGARET K., Johns Hopkins University.
DOBBELAAR, MARK E., Teacher of Science, Oradell High School.
FAHL, HELEN, Student, Oberlin College.
FERGUSON, FREDERICK P., Undergraduate Assistant, Wesleyan University.
FLEMING, ROBERT S., Science Critic Teacher, East Carolina Teachers College.
FRASER, LEMUEL A., Student, American University.
GALE, SHIRLEY, Radcliffe College.
GRAVES, E. IRENE, Senior Instructor in Biology, Bridgewater State Teachers College.
GRIFFITHS, RAYMOND B., Graduate Research Assistant, Princeton University.
HAINES, WILLIAM J., Wabash College.
HALL, LYDIA R., Graduate Assistant, Mount Holyoke College.
HAMANN, CECIL B., Assistant, Purdue University.
HARRIS, NELLIE R., Undergraduate Assistant, Montclair State Teachers College.
HOAGLAND, MARY, Swarthmore College.
JAEGER, LUCENA, Graduate Assistant in Zoölogy, University of Missouri.
JORDON, ELIZABETH L., Barnard College.
JOSEPH, SAMUEL, Student Laboratory Assistant, DePauw University.
KELLOGG, MARGARET P., Graduate Student, Cornell University.
KERRIGAN, SYLVIA, Graduate Assistant, University of Cincinnati.
LINSCHEID, ALFRED G., Western Reserve University.
LOVE, GENEVIEVE, Pennsylvania College for Women.
MCDONALD, BROWN, Laboratory Assistant, DePauw University.
MORRISON, PETER R., Swarthmore College.
NADLER, EVELYN R., Brooklyn College.
PIERSON, MARY E., Graduate Assistant, Mount Holyoke College.
REYER, RANDALL W., Cornell University.
ROLLASON, HERBERT D., JR., Middlebury College.
ROOT, CHARLOTTE C., Student, Mount Holyoke College.
RYAN, THOMAS I., Instructor, Boston College.
SACKETT, JOHN T., Graduate, University of Pennsylvania.
SANDERS, MARY ELIZABETH, Depauw University.
SCHAEFFER, BOBB, Graduate Student, Columbia University.
SCHNEIDER, MATHILDA E. C., University of Illinois.
SHEEHAN, ELEANOR L., Instructor, University of New Hampshire.
SMITH, RALPH I., Harvard University.

SNEDECOR, JAMES, Student, Iowa State College.
SPERRY, ROGER W., Oberlin College.
TABER, ELSIE, Instructor in Biology, Lander College.
TOWLE, HARRIET N., Assistant in Zoölogy, Wellesley College.
TROWBRIDGE, CAROLYN F., University of Iowa.
WARD, HELEN L., Assistant in Biology, Purdue University.
WELCH, D'ALTE A., Johns Hopkins University.
WELLS, LORNA A., Graduate Assistant, Oberlin College.
WILLIAMS, EDITH M., Student, Elmira College.

4. TABULAR VIEW OF ATTENDANCE

	1934	1935	1936	1937	1938
INVESTIGATORS—Total	323	315	359	391	380
Independent	222	208	226	256	246
Under Instruction	49	56	76	74	53
Research Assistants	52	51	57	61	81
STUDENTS—Total	131	130	138	133	132
Zoölogy	54	55	55	57	54
Protozoölogy	11	16	17	16	10
Embryology	30	33	34	35	34
Physiology	23	20	22	16	22
Botany	13	6	10	9	12
TOTAL ATTENDANCE	454	445	497	524	512
Less Persons Registered as Both Students and Investigators	15	16	24	13	16
	439	429	473	511	496
INSTITUTIONS REPRESENTED—Total	131	143	158	165	151
By Investigators	98	111	120	134	125
By Students	75	70	77	79	67
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	1	—	2	3	4
By Students	5	3	3	2	1
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	4	7	9	16	14
By Students	1	1	5	—	3

5. SUBSCRIBING AND COÖPERATING INSTITUTIONS IN 1938

American University
Amherst College
Barnard College
Belgian American Education Foundation, Inc.
Bowdoin College
Brothers College of Drew University
Brown University
Bryn Mawr College
Carnegie Institute of Washington
College of Physicians and Surgeons
College of William and Mary
Columbia University
Purdue University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
St. John's College
Smith College
Spring Hill College
State University of Iowa
Swarthmore College
Syracuse University
Temple University

Connecticut College
Cornell University Medical College
Dalhousie University
Dartmouth College
DePauw University
Duke University
Elmira College
General Education Board
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Iowa State College
Johns Hopkins University
Kenyon College
Eli Lilly & Company
Long Island University
Massachusetts State College
Memorial Hospital, New York City
Mount Holyoke College
New York State Department of Health
New York University
New York University Medical School
Northwestern University
Oberlin College
Pennsylvania College for Women
Princeton University
Toledo University
Tufts College
Union College
University of Chicago
University of Cincinnati
University of Illinois
University of Maryland Medical School
University of Minnesota
University of Missouri
University of Notre Dame
University of Pennsylvania
University of Pittsburgh
University of Rochester
University of Rochester Medical School
University of Vermont
University of Virginia
Vanderbilt University Medical School
Vassar College
Wabash College
Washington University
Washington University Medical School
Wellesley College
Wesleyan University
Western Reserve University
Wheaton College
Williams College
Wilson College
Yale University

6. EVENING LECTURES, 1938

Tuesday, June 21
Dr. E. H. Myers ..... "Life Cycle of Foraminifera."

Friday, July 1
Dr. M. H. Jacobs ..... "Blood and Zoölogical Classification."

Friday, July 8
Dr. S. O. Mast ..... "The Synthesis of Living Substance, as Exemplified in Chilomonas paramecium."

Friday, July 15
Dr. G. H. Parker ..... "The Color Changes in Animals and Neurohumoral Transmission."

Wednesday, July 20
Dr. Robert Chambers and
Dr. William Duryee ..... "Micromanipulation Studies on Cells and Nuclei."

Friday, July 22
Dr. O. E. Schotté ..... "Induction of Embryonic Organs in Regenerates and Neoplasms."

Friday, July 29
Dr. Eduard Uhlenhuth ..... "A Quantitative Approach to the Secretion Process of the Thyroid."

Friday, August 5
Dr. Robert Chambers ..... "Structural Aspects of Cell Division."

Tuesday, August 9
Dr. E. G. Conklin and
Dr. F. R. Lillie ..... "Informal Memorial of the Fiftieth Anniversary of the Founding of the Marine Biological Laboratory."

Friday, August 12
Dr. L. G. Barth ..... "Studies of the Factors Influencing Regeneration."

Friday, August 19
Mr. Columbus Iselin ..... "The Influence of Fluctuations in the Major Ocean Currents on the Climate and the Fisheries."

Friday, August 26
Dr. Peter Gray ..... "The Possibility of Affecting Developmental Patterns by Electrical Means."

Thursday, September 1 (Under the joint auspices of the Genetics Society of America and the Marine Biological Laboratory)
Dr. H. J. Muller ..... "The Remaking of Chromosomes."

7. SHORTER SCIENTIFIC PAPERS, 1938

Tuesday, July 5
Dr. W. H. Newton ..... "Endocrine Activity of the Placenta in Mice."
Dr. J. K. W. Ferguson and
Dr. H. O. Haterius ..... "Evidence for Hormonal Control of Uterine Motility by the Hypophysis in the Rabbit."
Dr. Roberts Rugh ..... "Experimental Studies on the Genital System of the Male Anuran."

Tuesday, July 12
Dr. G. W. Kidder and
Dr. C. A. Stuart ..... "The Rôle of Chromogenic Bacteria in Ciliate Growth."
Mr. J. A. Smith ..... "Some Effects of Temperature on the Reproduction of Chilomonas paramecium."
Dr. D. L. Hopkins ..... "Adjustment of the marine Amoeba, Flabellula mira Schaeffer, to changes in the Total Salt Concentration of the Outside Medium."
Mr. C. L. Claff ..... "Phenomena of Excystment in Colpoda cucullus."

Tuesday, July 19
Dr. K. C. Fisher and
Mr. R. Öhnell ..... "The Steady State Frequency of the Embryonic Fish Heart at Different Cyanide Concentrations."

Dr. Lena A. Lewis ..... "Studies on the Refractory State Resulting from the Repeated Injections of Adrenal Extract."
Dr. Emil Bozler ..... "Action Potentials of Visceral Smooth Muscles."
Dr. L. Irving ..... "Rhythmical Changes in Blood Flow Through Muscles."

Tuesday, July 26
Dr. B. H. Willier and
Dr. Mary E. Rawles ..... "Skin Transplants between Embryos of Different Breeds of Fowl."
Dr. Arthur Colwin ..... "Induction by Cauterization in the Amphibian Egg."
Dr. Viktor Hamburger ..... "The Innervation of Transplanted Limbs in Chick Embryos."
Dr. Paul Weiss ..... "The Effect of Mechanical Stress on Cartilage Differentiated in Vitro."

Tuesday, August 2
Dr. J. P. Visscher ..... "Some Recent Studies on Barnacles."
Dr. E. R. Jones, Jr. ..... "Observations on some of the Lower Turbellaria of the Eastern United States."
Dr. d'A. A. Welch ..... "Some Problems of Distribution and Variation in the Hawaiian Tree Snail Achatinella."

Tuesday, August 16
Dr. D. P. Costello ..... "Studies on Fragments of Centrifuged Nereis Eggs."
Dr. Victor Schechter ..... "Calcium and Magnesium in Relation to the Longevity of Egg Cells."
Dr. J. B. Buck and
Dr. R. D. Boche ..... "Some Properties of Living Chromosomes."
Dr. A. M. Lucas ..... "Some Cytological Studies on Virus-Infected Cells."
Dr. W. R. Duryee ..... "A Microdissection Study of Amphibian Chromosomes."

Tuesday, August 23
Mr. Karl Wilbur ..... "The Relation of the Magnesium Ion to Ultraviolet Stimulation in the Nereis Egg."
Dr. E. Eleanor Carothers ..... "Cytological Effects of X-Rays on Grasshopper Embryos."
Dr. J. Furth ..... "Quantitative Studies on the Effect of X-Rays on Mammalian Cells, and on the Mode of X-Ray Action."
Dr. P. S. Henshaw ..... "The Effect of X-Rays on Arbacia punctulata Sperm."
Dr. T. N. White ..... "Recovery of Arbacia Eggs from High Intensity X-Ray Effects."

8. GENERAL SCIENTIFIC MEETING, 1938

Tuesday, August 30

Miss A. M. Melland ..... "Isolation of Salivary Gland Nuclei."

Mr. Glenn H. Algire ..... "Cytological Studies on the Living Thyroid of the Salamander."

Dr. Ralph H. Cheney ..... "Micro-Structural Changes in Muscle Fibers after Caffeine."

Dr. Carl C. Speidel ..... "Some Features of Contraction Nodes and Retraction Clots as Observed in Single Fibers of Cardiac and Skeletal Muscle of Both Vertebrates and Invertebrates."

Dr. Michael J. D. White ..... "The Heteropycnosis of Sex Chromosomes and its Interpretation in Terms of Spiral Structure."

Dr. John P. Turner ..... "Mitochondria and other Inclusions in the Ciliate Tillina canalifera."

Dr. Robert Chambers ..... "Cytoplasmic Inclusions and Matrix of the Arbacia Egg."

Dr. M. J. Kopac ..... "The Devaux Effect at Oil-Protoplasm Interfaces."

Dr. M. H. Jacobs and
Dr. A. K. Parpart ..... "Further Studies on the Permeability of the Erythrocyte to Ammonium Salts."

Mr. A. J. Dziemian and
Dr. A. K. Parpart ..... "Permeability and the Lipoid Content of the Erythrocyte."

Mr. Louis-Paul Dugal and
Dr. Laurence Irving ..... "The Relation of the Shell to Anaerobic Metabolism in Venus mercenaria."

Dr. Alexander Sandow and
Dr. Kenneth Moritz ..... "Tension Output of Muscles in Hypotonic Solutions."

Dr. Dwight L. Hopkins ..... "The Mechanism for the Control of the Intake and the Output of Water by the Vacuoles in the Marine Amoeba, Flabellula mira Schaeffer."

Dr. N. S. R. Maloeuf ..... "On the Kidney of the Crayfish and the Uptake of Chlorid from Fresh Water by this Animal."

Dr. N. S. R. Maloeuf ..... "The Osmo-regulative Function of the Alimentary Tract of the Earthworm, and on the Uptake of Chlorid from Fresh Water by this Animal."

Dr. Ethel Browne Harvey ..... "Development of Half-Eggs of Chaetopterus Obtained by Centrifugal Force."

Dr. Paul S. Henshaw ..... "The Question of Whether the Delay in Cleavage of Arbacia Eggs Produced with X-Rays is Caused by a General Slowing of the Cleavage Process or by a Block at Some Particular Stage."

Mr. E. L. Chambers and
Dr. Robert Chambers ..... "The Resistance of Fertilized Arbacia Eggs to Immersion in KCl and NaCl Solutions."

Dr. Albert E. Navez ..... "Indolphenoloxidase in Arbacia Eggs and the Nadi Reaction."

Dr. K. C. Cole and
Dr. Howard J. Curtis ..... "Electric Impedance of Nerve During Activity."

Dr. Frank A. Geldard ..... "The Vibratory Response of the Skin and its Relation to Pressure Sensitivity."

Dr. E. Alfred Wolf ..... "Reversal of Phototropic Reaction in Daphnia by the Use of Photosensitizing Dyes."

Dr. Carl C. Speidel ..... "Motion Picture Showing Microscopic Changes in Fibers of Cardiac and Skeletal Muscle of Invertebrates and Vertebrates during Contraction, Retraction, and Clotting."

Dr. W. R. Duryee ..... "The Action of Direct Currents on the Cell Nucleus."

Dr. W. R. Duryee ..... "Hydration and Dehydration of Follicle Cell Nuclei."

Wednesday, August 31

Dr. Herbert Elftman ..... "The Function of Muscles in Locomotion."

Dr. William J. Bowen ..... "The Effects of Copper and of Vanadium on the Frequency of Division."

Dr. Sarah Bedichek ..... "Sex Balance in the Progeny of Triploid Drosophila."

Dr. Eduard Uhlenhuth,
Mr. James U. Thompson and
Mr. Joseph E. Schenthal ..... "The Antihormone Problems in the Salamander."

Dr. Roberts Rugh ..... "The Effect of the Sex-Stimulating Factor of the Anterior Pituitary Gland on the Testis of the Bullfrog."

Dr. J. Paul Visscher ..... "Studies on Barnacle Larvae."
Dr. Grace Townsend ..... "The Spawning Reaction of Nereis limbata with Emphasis Upon Chemical Stimulation."
Dr. Grace Townsend ..... "Physiological Assays Concerning the Nature of Fertilizin."
Dr. Elbert C. Cole ..... "A Study of the Integument of the Squid, During Staining with Methylene Blue."
Mr. Carl C. Smith and
Mr. Louis Levin ..... "The Use of the Clam Heart as a Test Object for Acetylcholine."
Dr. Oscar W. Richards and
Miss Katharine J. Hawley ..... "The Elimination of Molds."
Dr. S. E. Pond,
Mr. E. P. Little,
Mr. A. M. Smith, and
Mr. J. D. Graham ..... "A Comparative Study of Water Aspirators."

PAPERS READ BY TITLE

Dr. C. A. Angerer ..... "The Effect of Electric Current on the Physical Consistency of Sea Urchin Eggs."
Mr. C. W. J. Armstrong and
Dr. K. C. Fisher ..... "The Effect of Sodium Azide on the Frequency of the Embryonic Fundulus Heart."
Mr. Robert Ballantine ..... "Reducing Activity of Fertilized and Unfertilized Arbacia Eggs."
Dr. Ludwig von Bertalanffy ... "Studies on the Mechanism of Growth in Planaria maculata."
Mrs. Ruth Snyder Cooper ..... "Probable Absence of a Chromatophore Activator in Limulus polyphemus."
Mr. C. G. Grand ..... "Intracellular pH Studies on the Ova of Mactra solidissima."
Dr. W. R. Duryee ..... "The Action of Fixatives on the Isolated Cell Nucleus."
Dr. Adolph Elwyn ..... "The Melanophore-Expanding Activity of the Ascidian Neural Gland."
Mr. Richard W. Foster,
Mr. John D. Crawford and
Dr. Albert E. Navez ..... "Cardiac Rhythm in Pecten irradians (Lamarck)."
Dr. Stephen Karády ..... "The Alarm Reaction and Adaptation Syndrome in Lower Vertebrates (Fundulus majalis)."

Dr. M. J. Kopac ..... "Micro-estimation of Protein Adsorption at Oil-Protoplasm Interfaces."

Dr. M. J. Kopac and
Dr. R. Chambers ..... "Effect of the Vitelline Membrane on Coalescence of Arbacia Eggs with Oil-drops."

Dr. George Saslow ..... "The Osmotic Pressure of Gum Acacia Solutions."

Dr. A. A. Schaeffer ..... "Differences Between Scottish and American Amebas of the Species Chaos diffluens Müller."

Dr. Victor Schechter ..... "Induction in Griffithsia."

Dr. Victor Schechter ..... "Bacteria in Relation to Longevity of Egg Cells."

Dr. J. N. Stannard ..... "The Effect of Sodium Azide on the Respiration of Frog Muscle."

Dr. A. J. Waterman ..... "Respiratory Stimulants and Gastrulation in Arbacia."

Dr. Ralph Wichterman ..... "Does Transfer of Pronuclei ever Occur in Conjugation of Paramecium caudatum?"

Dr. E. Alfred Wolf and
Mr. A. S. Levenson ..... "Studies in Calcification. IV. A Contribution to the Problem of Skeletal Calcification in the Teleost, Fundulus heteroclitus."

Dr. Opal M. Wolf ..... "Mitotic Activity of the Islands of Langerhans and Parathyroids of Rats Following Pituitary Extract and Colchicine Injections."

Dr. Opal M. Wolf ..... "Oviducts of Pituitary Stimulated Females, Rana pipiens."

Miss R. A. Young ..... "The Effects of Roentgen Irradiation on Cleavage and Early Development in the Annelid, Chaetopterus pergamentaceus."

Mr. E. Zwilling ..... "The Effect of Perisarc Removal on Regeneration in Tubularia crocea."

DEMONSTRATIONS

Wednesday, August 31

Dr. Michael J. D. White ..... "The Spiral Structure of Animal Chromosomes."

Dr. P. S. Henshaw ..... "Cellular Abnormalities Produced by X-Rays."

Dr. K. S. Cole and
H. J. Curtis ..... "Electrical Impedance Changes in the Squid Giant Axon Following Excitation."

Dr. E. R. Clark and
Mrs. Eleanor Linton Clark ....a) "Marked Macrophages."
b) "Arterio-venous Anastomoses as Observed in the Living Mammal."
Mr. C. H. Norris ....."Method of Studying Elastic Tension of Marine Eggs."
Mr. G. H. Algire ....."Apparatus for the Cytological Study of the Thyroid in the Living Salamander."
Dr. J. P. Turner ....."Mitochondria and Other Inclusions in the Ciliate Tillina canalifera."
Mr. A. S. Levenson ....."Microscopic Sections through Head and Trunk Regions of Fundulus heteroclitus, Prepared by the Gömöre Silver Nitrate Method for the Study of Calcification."
Dr. A. K. Parpart and
Mr. S. B. Young ....."A Simple Glass Electrode System."
Dr. S. E. Pond and
Mr. E. P. Little ....."Water Aspirator Tests and Comparisons."
Dr. E. C. Cole .....a) "Methylene Blue Preparations of the Chromatophores of the Squid."
b) "A Low Voltage Lamp for General Microscopic Use."
c) "Methyl Methacrylate as a Mounting Medium for Macroscopic Preparations."
Dr. Grace Townsend ....."Spawning Reactions of Male Nereis limbata in Response to Glutathione."
Mr. C. C. Smith and
Mr. Louis Levin ....."The Use of the Clam Heart as a Test Object for Acetylcholine."
Dr. F. J. M. Sichel and
Dr. S. E. Pond ....."Multi Contact Rheotome."
Dr. Roberts Rugh ....."Urogenital System of the Male Frog Rana pipiens, Injected to Show the Course of Spermatozoa from the Seminiferous Tubules to the Wolffian Ducts."
Dr. P. S. Galtsoff ....."Sex Reversal in Adult Oysters."
Dr. P. S. Galtsoff ....."Method of Measuring and Recording the Rate of Flow of Water Through the Gills of the Oyster."
Dr. P. S. Galtsoff and
Mr. George Mishtowt ....."Respiration of the Oyster."

9. MEMBERS OF THE CORPORATION

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Maryland.
Billings, Mr. R. C., 66 Franklin St., Boston, Massachusetts.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Crane, Mr. C. R., New York City.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Massachusetts.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Massachusetts.
Jackson, Miss M. C., 88 Marlboro St., Boston, Massachusetts.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Massachusetts.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City.
Lee, Prof. F. S., College of Physicians and Surgeons, New York City.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Lowell, Mr. A. L., 17 Quincy St., Cambridge, Mass.
McMurrich, Prof. J. P., Toronto, Canada.
Means, Dr. J. H., 15 Chestnut St., Boston, Mass.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Mo.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, California.
Morgan, Mrs. T. H., Pasadena, California.
Morrill, Dr. A. D., Hamilton College, Clinton, N. Y.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
Sears, Dr. Henry F., 86 Beacon St., Boston, Massachusetts.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City.
Treadwell, Prof. A. L., Vassar College, Poughkeepsie, N. Y.
Trelease, Prof. William, University of Illinois, Urbana, Illinois.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, Calif.
Wilson, Dr. E. B., Columbia University, New York City.

2. Regular Members, 1938

Abramowitz, Dr. Alexander A., Biological Laboratories, Harvard University, Cambridge, Massachusetts.

ADAMS, DR. A. ELIZABETH, Mount Holyoke College, South Hadley, Massachusetts.

ADDISON, DR. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

ADOLPH, DR. EDWARD F., University of Rochester Medical School, Rochester, New York.

ALLEE, DR. W. C., The University of Chicago, Chicago, Illinois.

ALLYN, DR. HARRIET M., Mount Holyoke College, South Hadley, Massachusetts.

AMBERSON, DR. WILLIAM R., Department of Physiology, University of Maryland, School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.

ANDERSON, DR. E. G., California Institute of Technology, Pasadena, California.

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STUDIES ON THE TREMATODES OF WOODS HOLE

II. The Life Cycle of Stephanostomum tenue (Linton) [1]

W. E. MARTIN

(From DePauw University and the Marine Biological Laboratory, Woods Hole, Massachusetts)

This paper deals with the results of a study of a member of the trematode family Acanthocolpidae obtained during the summers of 1936 and 1938 at the Marine Biological Laboratory at Woods Hole, Mass. No previous experimental work has been done on the life cycles in this family, and consequently the systematic relationships have been in question. This paper throws some light on these problems. The results obtained may be of some economic importance because the adult members of this family are parasitic in marine fishes, several of which are food fishes.

A synopsis of this work was given before the American Society of Parasitologists at the 1938 meeting at Richmond, Virginia.

Historical

Some of the members of the family Acanthocolpidae were at first assigned to the old pseudogenus, *Distomum*, and, due to the presence of spines encircling the mouth, were thought to be related to the echinostomes. Nicoll (1915) placed some of the acanthocolpids in the family Allocreadiidae because of the similarity in the arrangement of the reproductive organs in the two groups. Winfield (1929) criticizes Nicoll's classification, stating, "The Stephanochasminae should be excluded (from the Allocreadiidae) because of the Y-shaped excretory bladder, the circle of head spines, and the armed cirrus and vagina." The family name, Acanthocolpidae, was created by Lühe in 1909 to include trematodes whose principal diagnostic characters are: a well-developed prepharynx and pharynx, a very short esophagus, a Y-shaped excretory bladder, the ovary in front of the testes, the uterus between the ovary and the ventral sucker, the cirrus and vagina armed with spines, and the genital opening medially located anterior to the ventral sucker. At present the following seven genera are included in the family: *Stephanostomum* Looss 1899, *Dihemistephanus* Looss 1901, *Deropristis* Odhner 1902, *Acanthocolpus* Odhner 1905,

[1] This work was made possible through the use of the laboratory facilities maintained by Purdue University at the Marine Biological Laboratory.

Acanthopsolus Lühe 1906, *Tormopsolus* Poche 1925, and *Echinostephanus* Yamaguti 1934. Because of the presence of connections between the excretory bladder and the ceca in the genus *Echinostephanus*, Yamaguti separated it from the genus *Stephanostomum*. However, McFarlane (1936) described such connections in *Stephanostomum casum* (Linton) and indications of them in *S. tristephanum*. This suggests that a more extensive and intensive study of this character is needed.

Reports of observations pertaining to the life cycles of members of this family have appeared from time to time. Lebour (1907) described a cercaria that developed in rediae in the limpet, *Patella vulgata*, which she believed to be the larval form of some member of the genus *Stephanostomum*. However, this cercaria lacked eyespots, had a long esophagus and a small sac-shaped excretory bladder, all of which were contrary to observations on the adult worms. The same author (1910) described a cercaria from *Buccinum undatum* which she thought was the larval form of *Acanthopsolus lageniformis*. This cercaria possessed eyespots and general characteristics which agreed with the structures of the adult. No experimental work was done to test the validity of her assumption. Some of the cercariae had tails while the majority did not, which, in conjunction with the absence of large glands in the body, was interpreted by Lebour to indicate that no second intermediate host was required. This seems questionable since Linton (1898), Stafford (1904), Lühe (1906), Nicoll and Small (1909), Nicoll (1910), and others have found metacercariae of this family in various species of fishes. Linton (1898) found cysts of *Distomum valdeinflatum* attached to the peritoneum of *Alutera schoepfi* and *Menidia menidia notata*. Stafford (1904) found the cysts of *Stephanochasmus histrix* on the fins of *Pseudopleuronectes americanus*. Lühe (1906) found *Stephanochasmus ceylonicus* encysted in the subcutaneous tissue of *Narcine timlei* taken off Dutch Bay, Ceylon. Lebour (1907) reported *Stephanochasmus metacercariae*, probably *S. baccatus*, under the skin of the dab, witch, and long rough dab. Nicoll and Small (1909) discovered the cysts of *Stephanochasmus baccatus* under the skin of *Pleuronectes limanda*. They state, "It is not at all improbable that the cercariae of *S. caducus*, *S. triglae*, and *S. baccatus* are all to be found encysted in young pleuronectid fishes." Nicoll (1910) reported finding cysts of *S. baccatus* in *Drepanopsetta platessoides*. Yamaguti (1934) found cysts of *Stephanochasmus sp.* with 46 collar spines in *Lotella physis* and *Engraulis japonica*, *S. sp.* with 36 collar spines in *Argentina kagoshimae*, and *S. sp.* with 54 collar spines in *Bothrocara zesta* and *Furcimarius sp.* He also found *Echinostephanus*

sp. with 40 collar spines encysted in the flesh of *Argentina kagoshimae.* The same author (1937) reported *Stephanochasmus bicoronatus* cysts in the body cavity of *Acanthogobius hasta* and on the gills of *Sciaena sp.* and *Taenioides lacepedi; Echinostephanus hispidis* cysts in the flesh of *Pseudorhombus pentophthalmus* and *Neopercis sexfasciatus* and *Tormopsolus* larvae encysted near the gills of *Leiognathus rivulata.*

Material and Methods

The snail, *Nassa obsoleta,* which serves as the first intermediate host, *Menidia menidia notata* the second intermediate host, and the puffer, *Spheroides maculatus,* which serves as the experimental definitive host, were all collected in the vicinity of Woods Hole. Naturally infected snails were used as sources of cercariae. Some *Menidia* and *Spheroides* were used for experimental feedings while others were retained as controls.

Living material was used in the study of many of the cercarial structures. Bouin's solution and a saturated aqueous solution of mercuric chloride were used as fixatives. Mayer's paracarmine was used to stain toto mounts, while sectioned material was stained with Ehrlich's hematoxylin. Infected snails, isolated in finger bowls filled with sea water, furnished a plentiful supply of cercariae for the experimental infection of *Menidia.*

Observations and Descriptions

The life cycle of *Stephanostomum tenue* involves the production of rediae and cercariae in the digestive gland of the marine snail, *Nassa obsoleta,* the development of the metacercariae in cysts in the liver of the small fish, *Menidia menidia notata,* and the maturation of the worm in the intestine of the puffer, *Spheroides maculatus.*

All measurements listed in this paper are expressed in millimeters.

The Redia (Figs. 3 and 4)

Natural infections of this trematode were found in about .4 per cent of the several thousand *Nassa obsoleta* under observation. Some increase in the number of infected snails in the latter part of the summer was noted, which may be correlated with the migratory habits of the hosts of the adult worms. The redia is an elongate, saccular structure with a pharynx and short rhabdocoel gut. The length of the gut, however, varies with age since it is nearly two-thirds the length of the very young redia (Fig. 4). The young redia also exhibits marked motility. The length of the redia varies from 0.14 to 0.66 with an average of about 0.5; the width varies from 0.03 to 0.14 with

an average of about 0.10. The pharynx varies from about 0.025 long by 0.028 wide to 0.052 long by 0.029 wide. The number of germ balls and cercariae per redia varies from 0 to 14 for the former and 0 to 5 for the latter. No ambulatory processes were present and no birth pore was observed.

The Cercaria (Fig. 1)

The cercaria is of the ophthalmoxiphidio type with a simple tail. In swimming the tail is lashed back and forth while the body is held almost straight. In finger bowls of sea water the cercariae swim about for a short time and then settle to the bottom to which they adhere by the tips of their tails. No special glandular bodies were found in the distal region of the tail that might account for this adhesive action. This behavior may be of importance in the completion of the life cycle since the cercariae may become attached to food particles and may be eaten by fishes. The cercaria exhibits a positive response to light.

The cuticula of the body is spinous with larger spines on the anterior end. In addition to the spines there are seven or eight setae projecting from each side of the body. These are irregularly spaced along the entire body length. The oral sucker bears two rows (of 21 each) of alternating large spines about 0.005 long. These spines are easily detached under even slight cover-glass pressure. The body length varies with the degree of contraction from 0.145 to 0.38 with an average of 0.24, while the body width varies from 0.045 to 0.086 with an average of 0.064. The tail averages about 0.183 long by 0.031 wide. The oral sucker averages about 0.031 long by 0.030 wide while the ventral sucker averages about 0.033 long by 0.030 wide. The ventral sucker bears two rows of small papillae with about 65 papillae in each row. Projecting anteriorly above the oral sucker there is a simple spear-shaped stylet about 0.014 long. The mouth

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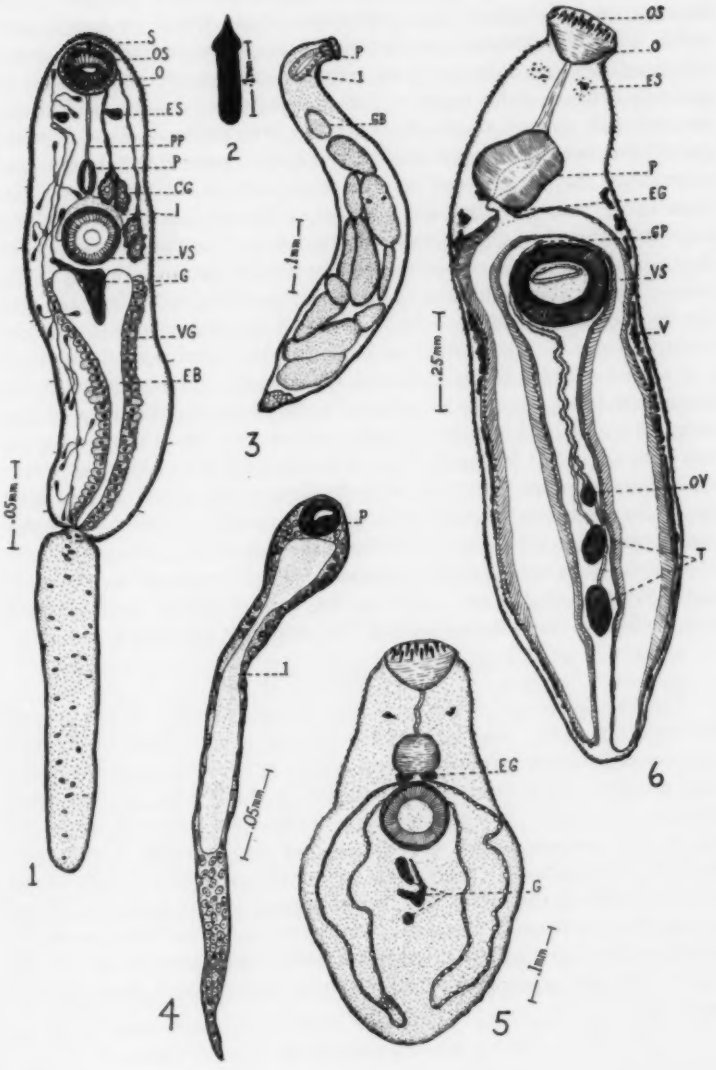
EXPLANATION OF PLATE

All drawings were made with the aid of a camera lucida. Abbreviations used: *CG*, cephalic gland; *EB*, excretory bladder; *EG*, esophageal gland; *ES*, eyespot; *G*, genital anlage; *GB*, germ ball; *GP*, genital pore; *I*, intestine; *O*, oral sucker; *OS*, oral spines; *OV*, ovary; *P*, pharynx; *PP*, prepharynx; *S*, stylet; *T*, testes; *V*, vitellaria; *VG*, vesicular gland; *VS*, ventral sucker.

FIG. 1. Ventral view of cercaria.
FIG. 2. Stylet of cercaria.
FIG. 3. Redia with germ balls and cercaria.
FIG. 4. Young redia showing elongate intestine.
FIG. 5. Metacercaria.
FIG. 6. Adult.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opens into a long narrow prepharynx approximately 0.038 long. The pharynx is subglobular and measures about 0.012 in length and width. The esophagus is extremely short. The rudimentary intestine branches just anterior to the ventral sucker and the branches do not extend beyond this organ. Two conspicuous eyespots are located, one on each side of the body, near the oral sucker. Four cephalic glands are located on each side of the body immediately lateral and anterior to the ventral sucker. On each side of the body the ducts from two glands pass anteriad median to the eyespot while the ducts from the other two glands pass anteriad lateral to the eyespot. The ducts of all four glands open to the exterior at the anterior end of the body. Other glands include numerous vesicular glands along the wall of the excretory bladder. The weakly Y-shaped excretory bladder extends almost to the ventral sucker. In some specimens the anterior wall of the bladder has a scalloped appearance. The main collecting ducts arise from the anterior margin of the excretory bladder and pass anteriad to the level of the eyespots where they bend on themselves and pass posteriad to supply both sides of the body. The flame cells are in seven groups of threes, with the first group given off just after the main duct bends posteriorly at the eyespot level. The other groups are given off at intervals along the side of the body.

The reproductive system is represented by a mass of deeply staining cells located just posterior to the ventral sucker and partially surrounded by the anterior wall of the excretory bladder.

The Metacercaria (*Fig. 5*)

The cercariae are taken into the digestive tract of the second intermediate host, *Menidia menidia notata*, where they work their way through the intestinal wall and encyst in the liver or mesenteries. No cercariae were observed to penetrate the bodies of the fishes through the skin. The metacercaria increases to several times the size of the cercaria. The 42 collar spines also increase in size until they are approximately 0.050 long. The eyespots and the glandular cells surrounding the excretory bladder undergo disintegration. There is a marked increase in the size of the pharynx. The branches of the intestine develop until they reach to near the posterior end of the body. The metacercaria is held within a rather tough, loose encystment sac.

The Adult (*Fig. 6*)

Nearly mature adult worms were obtained by feeding pieces of *Menidia* liver containing metacercariae to young puffers, *Spheroides maculatus*. The puffers were examined about two weeks after the

initial feeding and the worms were recovered from the intestine. Remnants of the eyespots were still present. The oral spines were the same in number and approximately of the same size as in the metacercaria. The relative proportions of the suckers and the pharynx were about the same as in the metacercaria. Advances in development over the conditions found in the metacercaria are: the differentiated testes and ovary located in the posterior one-third of the body, the reproductive tubes extending from these organs to the genital pore located on the mid-ventral side of the body immediately anterior to the ventral sucker, and the small clusters of vitelline cells extending along the sides of the body from the posterior end of the pharynx to near the posterior end of the body. Complete functional maturity of the reproductive systems had not been attained since no eggs had been produced.

The following measurements and description are based on but a few worms so that the range of variation is probably less than would be found with a larger number of individuals. Body length 1.9 to 2.2, width 0.5; oral sucker 0.13 long by 0.18 wide; ventral sucker 0.22 long by 0.25 wide; prepharynx from 0.19 to 0.31 in length by about 0.015 in width near the oral sucker to 0.031 at its widest point near the pharynx; pharynx about 0.22 long by 0.16 to 0.18 in width; esophagus 0.04 to 0.07 long; ovary about 0.057 long by 0.03 to 0.038 wide; anterior testis 0.10 to 0.136 long by 0.04 to 0.07 wide, posterior testis 0.09 to 0.14 long by 0.04 to 0.07 wide.

Linton (1898) described *Distomum tenue* from the rectum of the striped bass, *Roccus lineatus*, collected at Woods Hole. The description he gave, with measurements in millimeters, is as follows: oral spines 0.051 long by 0.018 wide at base; esophagus 0.44 long by 0.34 wide (he undoubtedly has used the term esophagus for the pharynx); vitellaria voluminous, peripheral in the posterior region; genital aperture immediately in front of the ventral sucker; ova not numerous and comparatively large, lying close behind the ventral sucker; ova length 0.088, width 0.044; body length 2.9, width 0.28; diameter of oral sucker 0.26, of ventral sucker 0.38.

Discussion and Conclusions

Most descriptions of the adult members of this family show them to have remnants of eyespots. This may indicate that the family represents a fairly compact, closely related group. When the excretory bladder is mentioned at all in the descriptions of species, it is described as Y-shaped. However, in my study of living specimens of *Deropristis inflata*, a simple tubular or sac-shaped bladder was found.

There is very little in the literature on the rest of the excretory system although Pratt (1916) in his description of *Stephanochasmus casum* showed that the main collecting tubes pass anteriad to near the level of the eyespots without giving off secondary tubes.

The arrangement of the reproductive organs in the family Acanthocolpidae, as was pointed out by Nicoll (1915), is similar to the arrangement of these organs in the family Allocreadiidae. There is also some suggestion of similarity in the excretory systems of these two groups. In addition, the members of both families are primarily parasites of fishes. This suggests a rather close relationship between the two families. However, the elucidation of the life cycles of other genera is needed before a positive statement can be made.

The family Acanthocolpidae seems to be cosmopolitan in distribution since some of its members have been found in European, Greenland, North American, Japanese, and Ceylonese waters.

There has been some confusion in the literature concerning the generic name *Stephanostomum*. This confusion resulted from Looss' first (1899) naming the genus *Stephanostomum* and then changing it to *Stephanochasmus* (1900) because of its similarity to the genus *Stephanostoma* Danielson and Koren, a genus of Gephyrean worms.

Summary

It was found that the life cycle of *Stephanostomum tenue* involves the development of rediae and cercariae in the marine snail, *Nassa obsoleta*, the utilization of the small fish, *Menidia menidia notata*, as the second intermediate host, and the development of the adult worm in the intestine of the puffer, *Spheroides maculatus*. Although the puffer may serve as the experimental definitive host, the striped bass, *Roccus lineatus*, is probably a natural one.

About .4 per cent of the *Nassa obsoleta* observed were infected with this parasite.

The excretory system of the cercaria is represented by the formula 2[3+3+3+3+3+3+3].

The arrangement of the reproductive organs, some similarity in the excretory systems, and the fact that fishes serve as hosts to the adult worms suggest an affinity of the Acanthocolpidae to the family Allocreadiidae.

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AN HERMAPHRODITE ARBACIA

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Among the many thousands of *Arbacia punctulata* opened in the course of ten summers at Woods Hole, and many hundreds of *Arbacia pustulosa*, *Sphaerechinus granularis*, *Paracentrotus lividus* and *Parechinus microtuberculatus* opened during several springs at Naples, and many hundreds of *Strongylocentrotus dröbachiensis*, from Maine, I observed last summer for the first time an hermaphrodite sea urchin, an *Arbacia punctulata*, opened on July 4, 1938. One other case of hermaphroditism in *Arbacia punctulata* has been described by Shapiro (1935); it was found late in the season of 1934 at Woods Hole. His animal had four testes and one ovary. It was fertile *inter se*, and all the eggs formed fertilization membranes, but the cleavages were delayed and abnormal. Many blastulae were obtained and 30 per cent gave rise to gastrulae; there was apparently no further development. James Gray (1921) described a *Strongylocentrotus lividus* in which three of the gonads were completely female, another almost completely so and the fifth contained both eggs and sperm which were fertile *inter se;* development of the eggs is not described. Gadd (1907) described a case of hermaphroditism in *Strongylocentrotus dröbachiensis* at the Mourmanschen Biological Station which had four female gonads and one male, but he does not give any details. The above are the only recorded cases of hermaphroditism in sea urchins, and it is indeed a rare phenomenon.

The hermaphrodite *Arbacia* which I found last summer was quite normal in external appearance and of average size. On removing the ventral portion of the shell, as usual in preparing the eggs, the gonads looked normal except that four were red ovaries and the fifth a white testis with sperm oozing out. Photograph 1 is of the five gonads immediately after removal to sea water. Microscopic observation of the living gonads showed that none of them was entirely male or female. The ovaries had a few tubules containing sperm and the testis contained some ova in various stages of development; that is, the gonads were really ovo-testes but predominantly female or male. A portion of a gonad, living and unstained, is shown in Photograph 2; the ovarian tubules are dark (from the red pigment) and the testis tubules are light with scattered pigment spots; a few eggs have been liberated and lie free in the space between the tubules. A stained

EXPLANATION OF PLATES

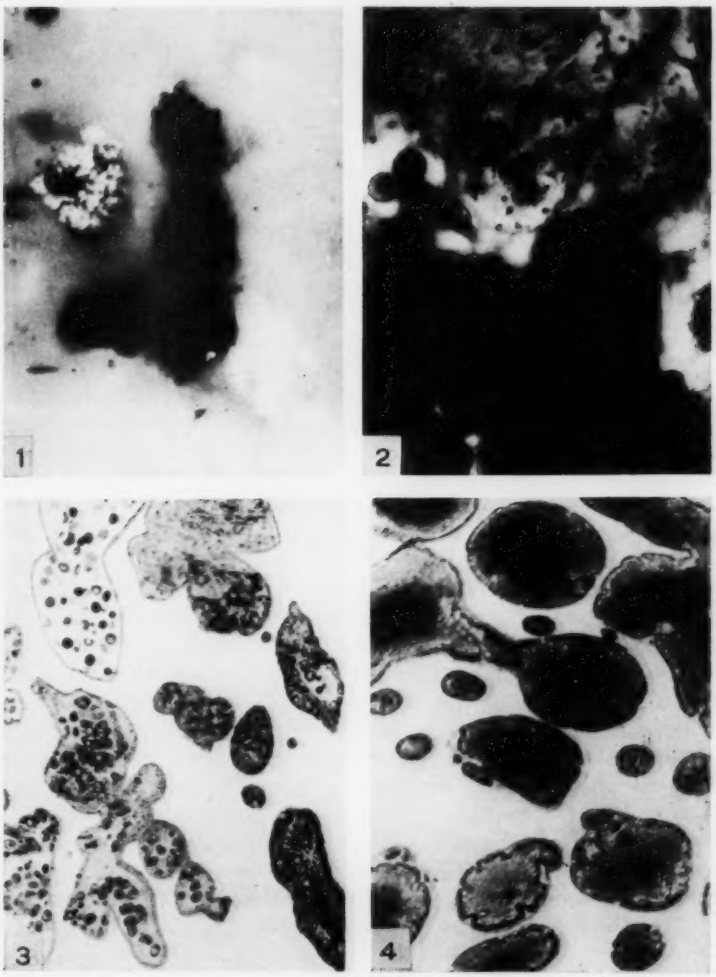

PLATE I

PHOTOGRAPH 1. Gonads of the hermaphrodite *Arbacia*, immediately after removal from the shell; one testis (white) and four ovaries (black). Note the small piece of testis (white) at edge of the lower right ovary.

PHOTOGRAPH 2. Part of a living gonad, showing testis tubules (white with pigment spots) and ovarian tubules (black) containing eggs, as seen under the microscope. A few eggs are seen free in the space between the tubules.

PHOTOGRAPH 3. Prepared stained section of a gonad predominantly female containing eggs in various stages of maturity. One testis tubule is seen at lower right.

PHOTOGRAPH 4. Prepared stained section of a gonad, predominantly male, containing mostly ripe sperm. One ovarian tubule is seen at lower right.

section of a predominantly female gonad is shown in Photograph 3; all the tubules are filled with eggs in various stages of development except the lower right which is mostly testis. Photograph 4 is a section of the predominantly male gonad; the tubules are all filled with sperm except one at the lower right which contains eggs. Photograph 5 is a section of a predominantly female gonad showing greater detail. Sections of normal ovaries and testes are exactly like these except that there is no mixture. As far as I could tell, especially from a study of the living gonads, the eggs and sperm in the hermaphrodite gonad are separate in the small tubules, and do not lie together without any partition. The eggs are not fertilized until they have been liberated from the tubules into the sea water, probably because the sperm are not motile until in sea water. As soon as the eggs have poured out from the tubules into the sea water, they are immediately fertilized by the sperm which have poured into the sea water and become active. At any one time, therefore, the fertilized eggs are found in various stages of development.

The eggs of the hermaphrodite are perfectly fertile with its own sperm. Normal fertilization membranes are formed, first cleavage takes place normally and at the normal time, and the later cleavages also, and practically all the eggs develop. The only unusual phenomenon was the occurrence of giant eggs. These were about 1 per cent of the total and were all of the same size, 96 μ in diameter, giving a volume of 463,000 μ^3 whereas the normal egg has a diameter of 74 μ and a volume of 212,000 μ^3; the giant eggs are approximately twice the normal volume. The origin of the giant eggs is not known, but they do not arise from fusion of ripe eggs since giant immature eggs also occur. I have found similar giant eggs in other *Arbacias* but very rarely, and I have also found in another *Arbacia* normal-sized eggs with giant nuclei. These nuclei measured 25.6 μ in diameter giving a volume of 8,785 μ^3, whereas the normal nucleus measures 11.5 μ in diameter, giving a volume of 796 μ^3; the giant nuclei are thus about eleven times the normal volume.

Eggs in late cleavages (3½ hours, and less, after fertilization at

PHOTOGRAPH 5. Prepared stained section of a gonad under higher magnification to show greater detail. The gonad was predominantly female, but the portion photographed predominantly male.

PHOTOGRAPH 6. Self-fertilized eggs 3½ hours (21° C.) after opening the animal. Most of the eggs are in late cleavage stages, but some are not so far advanced since they have come from the tubules and been fertilized later than the others. Note the giant eggs, also developing normally.

PHOTOGRAPHS 7–9. Normal development of self-fertilized eggs.

PHOTOGRAPH 7. Very early pluteus, self-fertilized, 31 hours old.

PHOTOGRAPH 8. Pluteus, self-fertilized, 35 hours.

PHOTOGRAPH 9. Pluteus, self-fertilized, 48 hours.

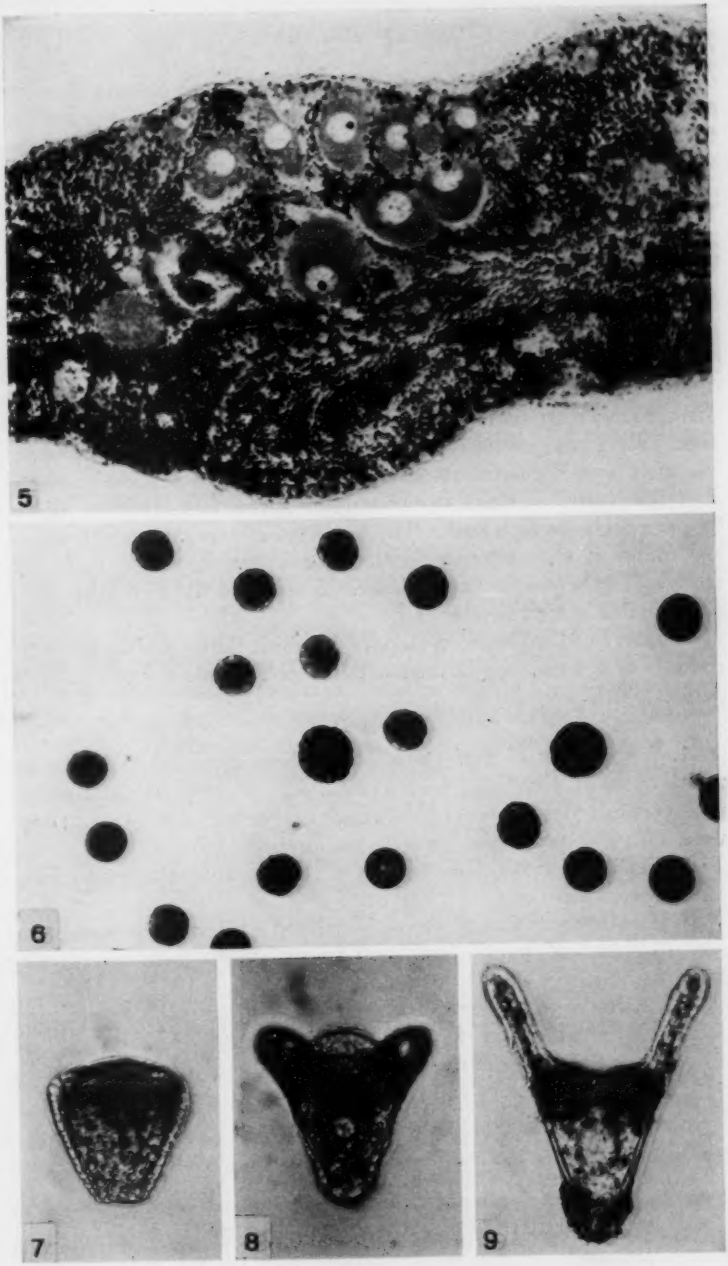

PLATE II

21° C.) are shown in Photograph 6, and one may observe here the giant eggs. The eggs, including the giant ones, develop quite normally and become swimming blastulae at the normal time, 9 hours after fertilization. The blastulae develop into perfectly normal plutei (Photographs 7–9), and these were kept for nine days. The plutei from the giant eggs were indistinguishable from the others which vary greatly in size according to age.

The sperm were perfectly normal in fertilizing other eggs as well as the hermaphrodite eggs (98 per cent), and the eggs from the hermaphrodite could be fertilized perfectly well by sperm from another sea urchin. This latter fact was ascertained by putting a small part of an ovary into fresh water for about ½ minute to kill the sperm on the outside; then the ovary was transferred to sea water. After an hour, only 1 per cent of the eggs shed were fertilized (by a few sperm liberated from the ovotestis after washing). But when the shed eggs were transferred to sea water containing sperm from another animal, 98 per cent were fertilized. The fertilization was therefore made by the sperm of the normal animal. These eggs developed quite normally. The hermaphrodite animal is therefore fertile with other males and females as well as *inter se*.

I think this the first recorded case in which the eggs of an hermaphrodite sea urchin, self-fertilized, developed absolutely normally to perfect plutei.

Summary

1. A rare case of perfect functional hermaphroditism in the sea urchin *Arbacia punctulata* is described. There were four red gonads predominantly female and one white gonad predominantly male; there were a few tubules of the opposite sex in all the gonads.

2. Fertilization occurred as soon as the sexual products were liberated in sea water.

3. The development of the self-fertilized eggs was absolutely normal, in time and morphology, and normal plutei were raised, nine days old.

4. There occurred about 1 per cent of giant eggs; these were twice the normal volume, and they also developed normally.

5. Both the eggs and the sperm also functioned perfectly normally with other normal males and females.

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KARYOKINESIS DURING CLEAVAGE OF THE ZEBRA FISH BRACHYDANIO RERIO

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Introduction

The results presented in this paper have been obtained in the course of a comprehensive study on the periodicity of cell division and mitotic rate during development. A discussion of these results, confined to observations on teleosts, is a necessary preliminary to the more complete investigation, with history and literature, to be presented later.

To obtain a definite picture of the rôle of cell division in development, it is necessary to determine not only the number of mitoses which occur at given times and at given places, but also the duration of a single mitosis and the manner in which it proceeds in different stages of development. In spite of many careful investigations on the rate of mitosis, the duration of mitosis at different periods of development has not been sufficiently determined.

The most successful method in investigating the rôle of cell division in development has been that of Richards (1935) and others who tried to determine mitotic activity by means of a mitotic index or the percentage number of dividing cells. However, it is not possible to tell by this method whether mitoses occur at periodic cycles or are evenly distributed, so that the counting at any time will actually furnish a figure which approximates the average mitotic rate. The present paper deals with the manner and duration of mitosis only during cleavage.

The egg of the zebra fish (*Brachydanio rerio*), recently described as a favorable laboratory subject (Roosen-Runge, 1938), is especially adapted to this study, because of its rapid development, its transparency, and more particularly because the cell nuclei can be easily observed in the living egg. Three lines of investigation will be described in this paper, namely, (1) the morphology of the living and of the fixed nuclei; (2) the duration of divisions and of mitotic phases; and (3) their reaction to temperature changes.

Materials and Methods

Information concerning the propagation and raising of the eggs of the zebra fish may be found in an earlier paper (Roosen-Runge). For

observation of the living egg, a slide with a covering about 1 mm. thick of a mixture of bee's wax and paraffine was used. A hole, the diameter of the egg, was then cut through the layer of wax in order to let the light come through, with a glass ring, 22 mm. wide and 9 mm. high, added to prevent currents from moving the egg. The slide was immersed in water in a large dish of about 150 cc. capacity, to insure an abundant oxygen supply. The egg was then oriented in the hole and all observation carried on, with the slide so immersed, by means of a water-immersion lens (Zeiss, × 40), having sufficient depth of focus to make visible the cells inside the cell membrane. Although the use of an oil-immersion lens is also feasible, it is only useful to check up on details which on the whole can be seen just as clearly with the water-immersion lens.

The temperature was regulated with an ordinary desk lamp shining from varying distances upon the observation dish. This simple device proved sufficient to keep the temperature constant within the range of half a degree Centigrade, since the amount of water in which the egg was kept, being fairly large, made it possible to control the temperature almost continuously during the period of development. Thus the eggs continued to develop under the microscope without the least sign of disturbance from the beginning of the second to the end of the tenth cleavage, that is, for a period of about three hours.

Bouin's solution was used for fixation. The egg membrane and in most cases the yolk were removed after fixation, for it is then quite easy to tear off the membrane from the hardened egg and to remove the brittle yolk. Dioxan or alcohol + benzol was used for dehydration, but the former is the simpler method and, therefore, to be preferred. All sections were stained in Heidenhain's haematoxylin and cut 6 or 8 microns in thickness.

Morphology of the Nuclei

It is impossible to study the nucleus in the living egg before the first cleavage since the delicate structure is then hidden by coarse granules which are whirled up at the base of the cell by the streaming of the protoplasm into the blastodisc. During the first cleavage the streaming still continues, offering some difficulties to the observer. Accurate observation of the nucleus becomes possible only when the cytoplasm clears at the end of the first cleavage. The two nuclei appear as ovals with a longitudinal axis of approximately 18 μ. The outlines are fine and smooth. Two or three, sometimes more, very delicate curved lines divide the nucleus into several sections (Fig. 1). The first signs of mitosis are the swelling of the nucleus and the

appearance of irregularities in its oval shape when tiny indentations can be seen at the poles which appear flattened so that the nucleus assumes a barrel-like shape. Short rays which point toward the center of the nucleus seem to radiate from the depths of the indentations. Very often the nucleus appears to be divided lengthwise into halves by a fine channel which is filled with some substance a shade darker than the nuclear sap.

In the living egg the appearance of the indentations marks the beginning of a very rapid disintegration of the nuclear membrane. The whole circumference appears strongly wrinkled and rapidly fades out, together with the partition lines inside of the nucleus. In a short time no traces of nuclear structures are left. By watching very closely, one can for a moment fancy where the nucleus has been, because this area appears somewhat lighter and free from the tiny granules which are a part of the cytoplasm throughout the cell. Before nuclear structures become visible again, the cell almost completes its division. The changes in the cytoplasm and the shape of the cell during mitosis have already been described (Roosen-Runge, 1938).

Sometime after the furrow has completely cut through, there appears in the center of each daughter cell a group of tiny dark granules. These granules represent the chromosomes. They swell, become lighter, and finally appear as little circles or vesicles with very distinct outlines. The vesicles go on swelling rapidly until they come into contact with each other, eventually forming one body with a common but irregularly curved contour. The outlines of the individual vesicles remain visible for a time, some of them fading out finally, while others do not disappear until the breakdown of the nuclear membrane in the next prophase.

Observations on the living nuclei confirm some of the results obtained from sectioned material. The outstanding feature in the karyokinesis of the teleost blastomeres is the formation of chromosomal vesicles during the telophase. These chromosomal vesicles are quite commonly found in early development and are supposed to persist through the interkinetic phase into the prophase. This interpretation has been made very probable by A. Richards (1917) and B. G. Smith (1929) from the study of sections. It can be proved by the study of living nuclei, in which some of the walls of the vesicles can actually be seen to persist in the interphase nucleus. Some of the walls, however, do not remain visible, but this seems to be due to their thinning out and not to their complete disappearance, since the sections also show some partitions, very dark and distinct, while others are delicate and inconspicuous. In many instances the sectioned nuclei can be seen

divided into halves, inside of which the vesicles are visible. The halves are separated by a gap, apparently filled with cytoplasm, which corresponds to the observations on the living nuclei. The halves represent the paternal and the maternal parts of the chromosome set, as first described by Moenkhaus (1904) in teleost hybrids, and by many early workers on other forms.

How the vesicles arise from the anaphase chromosomes and how the chromosomes are formed from the vesicles in the prophase, cannot be determined accurately from fixed material, nor do observations of the living nuclei solve any of these problems. Richards (1917) concluded that the vesicles are formed by a swelling of the chromosomes so that finally the walls contain the chromatin material and enclose a space "filled in from the fluid portion of protoplasm." Smith (1929), on the other hand, studied the karyokinesis in *Cryptobranchus* eggs and found that the vesicular membrane was of cytoplasmic origin, developed under the influence of the chromosome within. Pictures like those of Smith certainly cannot be seen in sections of either *Fundulus* or *Brachydanio* eggs. The study of living nuclei only confirms the impression that the chromosomes actually swell during the telophase and that the vesicular wall represents the surface of the chromosome rather than a structure formed *de novo* from the cytoplasm. My own material does not show some of the details as distinctly as they appear (according to Richards) in *Fundulus*, although the formation of the vesicles and their persistence through the interphase could be clearly seen in the sections as well as in the living egg. Nevertheless, the behavior of the chromosome material still remained puzzling. That the reader may be better able to appreciate its actual appearance, I have used photographs (see plate) rather than drawings, as Richards and others have done. Attempts at drawing present possibly too great a temptation to express a prejudiced interpretation not justified by the actual material.

The prophase stage in the karyokinesis of the living blastomere has already been described. The appearance of the nucleus as a whole corresponds very well with the observations of the sections. Because of the rapidity with which the chromosomes reappear and arrange themselves, only a few figures in these phases will be found in material fixed at random. However, by closely watching the living nuclei and taking into account the time necessary for sufficient penetration of the fixing fluid to arrest the mitosis (about half a minute for Bouin's fluid), it is possible to fix material in any desired stage. It can then be seen that the individual chromosomes become clearly visible only immediately before the breakdown of the nuclear membrane. They seem to begin

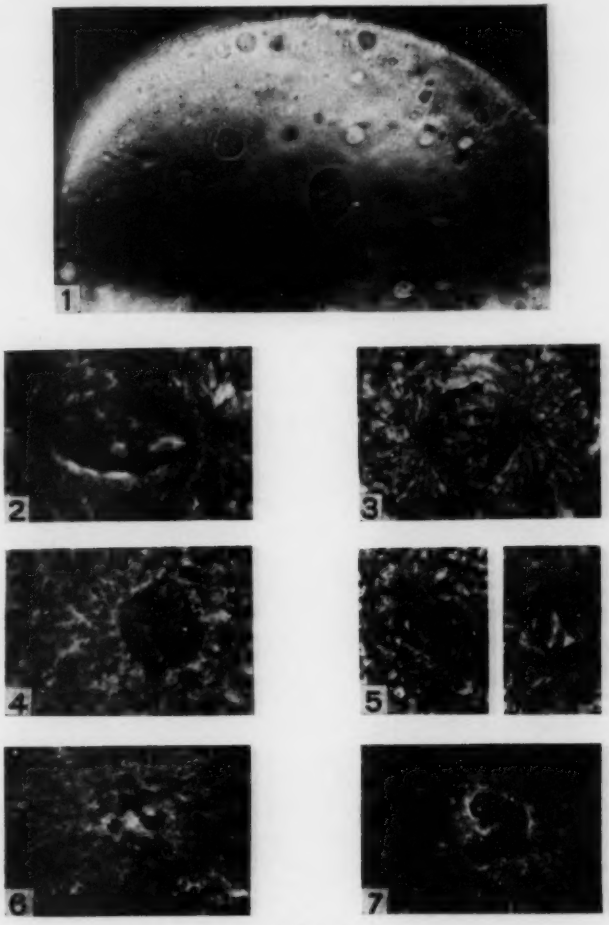

PLATE I

EXPLANATION OF FIGURES

FIG. 1. One of the eight blastomeres of a zebra fish egg, living. Nucleus with a few partition lines within, in the center. × 500.

FIGS. 2–7. Nuclei, fixed in Bouin's, Heidenhain's haematoxylin, 6–8 μ. × 1100. The different sizes of the nuclei are due to their belonging to different cleavage stages.

FIG. 2. Prophase in the beginning. Vesicles still visible. Indentations at the poles.

FIG. 3. Advanced prophase, chromosomes appearing.

FIG. 4. Chromosomes forming metaphase plate. Outline of nucleus still visible.

FIG. 5. Nuclei from cells after the twelfth cleavage, showing typical spireme formation in the prophase.

FIG. 6. Early telophase. Formation of chromosomal vesicles.

FIG. 7. Late telophase. Vesicles in contact. Paternal and maternal half of chromosomes apparently separate.

the arrangement into a plate while still inside of the membrane (Fig. 4). This fact has been confirmed by observations on the living eggs of another teleost, *Epiplaty chaperi*, in which the chromosomes are somewhat more easily discernible in life. Directly after the breakdown of the nuclear membrane the chromosomes can be seen arranged in a metaphase plate but very soon afterwards they begin separating. This observation shows that the disappearance of the membrane actually occurs relatively late. Individual chromosomes inside the separate vesicles, as pictured by Richards, could not be found in sections of the zebra fish egg.

The Duration of Cell Division

The absolute duration of cell division varies tremendously in different animals, and in different cells of the same animal, despite the fact that karyokinesis is supposed to occur essentially in the same way in all of them. The duration of mitosis is characteristic for the different kinds of cells. It can only be measured accurately through the direct observation of living material. The relative time of mitotic phases has been estimated by using the percentage number of cells active in the different stages, but in many cases, as will be pointed out later, this method is very erroneous. It seems, therefore, that direct observation is the safest method for determining the relative intervals in cell division.

The most considerable error in measuring the duration of cell division in life arises from the difficulty in finding any definite point of departure. Neither the beginning of the prophase nor the last stage of the telophase can be defined accurately, so that only a very few events are established sharply enough to serve as marks by which stages may be measured. In the blastomeres of the zebra fish the swelling of the nucleus at the beginning of the prophase, the breakdown of the nucleus, the appearance of the furrow, the completion of the furrow, the first appearance of the chromosomes in the telophase, and finally the completion of the rounded nucleus, furnish seven criteria of very different value. The time of the formation of the furrow, which means the division of the cytoplasm, can only be used indirectly for the determination of karyokinetic stages, although it may serve to subdivide the interval in which nuclear structures cannot be observed at all. The moment when the nucleus seems completely rounded and smoothly outlined is almost impossible to define, and its determination involves a considerable error. The swelling of the nucleus in the early prophase is also difficult to observe, but it is possible to determine its approximate beginning somewhat better with the aid of a micrometer

eye-piece, the scale of which will permit accurate observation of small changes in size. The reappearance of the chromosomes as tiny granules in the telophase is an event more easily determined. Under favorable conditions it is quite possible to watch the optically empty central area of the cell and to see the chromosomes become visible. I estimate the possible error under optimal conditions to be not more than 30 seconds or 3 per cent of the whole time of cleavage. By far the best mark, because of its rapidity of occurrence, is the breakdown of the nucleus. The nuclear membrane not only disappears in from 15 to 30 seconds, but the onset of this event is foreshadowed by a series of preparatory events, namely, the swelling of the nucleus and the wrinkling of the membrane, which makes it possible to predict the time of breakdown quite accurately. The error in determining the precise time of this occurrence is certainly not greater than 15 seconds, which is about 1.5 per cent of the whole time of cleavage. We have thus found two marks which seem reliable, because their errors can be estimated with considerable accuracy at only 1.5 to 3 per cent of the entire duration of cleavage. All other marks certainly have a higher error in determination, and if they are to be used for an estimate of the duration of the mitotic phases, this uncertainty has to be kept in mind.

The time for each cleavage from the first to the tenth is almost the same in different eggs, provided that a constant temperature is maintained and the oxygen supply is sufficient. During the process of cleavage the cell divisions follow each other without a typical resting stage, therefore the cleavage time was measured from the breakdown of the nucleus to the breakdown of the daughter nuclei. In Table I the results are compared with those of Jordan and Eycleshymer (1894) on amphibian blastomeres. The numbers concerning the zebra fish egg are all averages of at least 10 eggs. It can be seen from Table I that in every species the divisions show a characteristic duration. In four of the six animals the divisions show a trend towards acceleration before they finally begin to slow down. (The more complicated curve for the *Amblystoma* egg cannot be discussed here.) The turning point for this trend comes at different times. In the egg of the zebra fish the acceleration is at its height during the fifth cleavage. It seems significant that this is the last division when only one cell layer is involved, for the sixth cleavage is horizontal and divides the blastoderm into two layers. The sixth cleavage takes a slightly longer time than the preceding division, and from then on the process of cleavage gradually becomes slower and slower. Acceleration and retardation seem to involve the whole mitotic process uniformly and not any of its

phases differentially. Only during the ninth and tenth cleavages has a prolonged interkinetic phase been recorded, but as the error in determining this phase is even greater than for any other, no conclusion can be drawn from observations made at these stages of development.

TABLE I

Duration of cleavage divisions in amphibian and teleost eggs.* The times enclosed in brackets refer to individual cases and are not averages.

Temperature, ° C.	*Amblystoma punctatum*	*Rana palustris*	*Diemectylus viridescens*	*Bufo variabilis*	*Epiplatys chaperi*	*Brachydanio rerio*
	18	18	18	18	24	25
Duration of Cleavage Divisions						
Fertilization to first cleavage	10 hrs.?	4–5 hrs.	10 hrs.	4–5 hrs.		25 min.?
First to second cleavage	1 hour 50 min.	1 hour 15 min.	2 hrs.	1 hour 5 min.	2 hrs. 2 min.	20 min.
Second to third cleavage	1 hour 55 min.	1 hour 15 min.	1 hour 45 min.	1 hour	44 min.	19½ min.
Third to fourth cleavage	2 hrs.		1 hour 40 min.	1 hour	(43 min.)	19 min.
Fourth to fifth cleavage	1 hour 40 min.		1 hour 50 min.	1 hour	(41 min.)	18 min.
Fifth to sixth cleavage	(1 hour 35 min.)		(2 hrs. 45 min.)		(39 min.)	17½ min.
Sixth to seventh cleavage	(1 hour 25 min.)		(2 hrs. 45 min.)		(39 min.)	18½ min.
Seventh to eighth cleavage	(1 hour 25 min.)		(2 hrs.)		(40 min.)	19 min.
Eighth to ninth cleavage	(1 hour 25 min.)					20½ min.
Ninth to tenth cleavage						20 min.

* The data on amphibian eggs are taken from Jordan and Eycleshymer (1894).

In measuring the relative duration of the mitotic phases every cleavage can, of course, be observed. Most observations, however, were made during the sixth to ninth cleavages, since these stages had to be studied also for the periodicity of divisions, which will be discussed later. The arbitrary definition of the stages is obviously

a matter of terminology so long as the fundamental mechanism of mitosis is not understood.

The prophase was defined as extending from the first swelling of the nucleus until the break-up of the membrane. The time from the break-up until the chromosomes reappeared was assumed to be the duration of the metaphase plus the anaphase. As to the duration of both of these phases, it can only be stated that the metaphase is much shorter than the anaphase. This is true for two reasons, namely: (1) Nuclei which were observed up to the breakdown of the membrane and then immediately fixed always showed the chromosomes already slightly apart, and (2) the very rapid passing of the metaphase as described can actually be seen in the egg of *Epiplaty chaperi*. From these observations it must be concluded that the metaphase probably takes not much longer than one minute, or about 5.5 per cent of the total division time.

TABLE II

Duration of mitosis and mitotic phases.

Material	Total duration	Prophase	Metaphase	Anaphase	Telophase	Author
	minutes	*per cent*	*per cent*	*per cent*	*per cent*	
Protozoön: *Rhagostoma schüssleri*	32.5	18.5	12.5	18.5	53.5	after Darlington
Protozoön: *Euglypha sp.*	179	22.5	14.0	3.5	60.0	after Darlington
Erythrocytes, *Triton*	150			40.0	46.5	Jolly
The same	180	19.5	12.0	19.5	50.0	Wassermann (after Jolly)
Chorioidea, cartilage in chicken, culture	35	20.0		80.0		Strangeways
Blastomeres, *Brachydanio*	16	18.5		55.5	26.0	
The same, interkinetic phase counted as telophase	18	16.5		50.0	33.5	Roosen-Runge

In Table II, some results on the relative duration of mitotic phases in various animals have been compared. They were all obtained by direct observation. Interesting data like those of Lewis and Lewis

(1917) have been omitted since they are given too inaccurately for the present purpose. They seem, however, not to be in general disagreement with the figures presented here. The significance of the data compared lies in the fact that they agree surprisingly well, in spite of the different kinds of material used by the different investigators as well as the great disparity in the observations made with relation to the total duration of mitosis and the definition of its phases.

The relative time for the prophase varies only from 18.5 to 22.5 per cent in cells as different as those of protozoa, chicken cartilage, and fish blastomeres. The reported times of the metaphase vary also only slightly. However, in both the anaphase and the telophase there is considerable variation although it is smaller in the anaphase than in the telophase.

TABLE III

The duration of cleavage divisions under different temperatures.*
The times are minutes.

Cleavage	2	3	4	5	6	7	8	9	10
23° C.						(21)			
23½		(20)	(20)			(21)			
24						20	19.5		
24½	21		19	(20)	(19)				
25		19.5	18.5	18.5	18	18.5	19	19.5	20
25½			(18)	18	17	17	18		
26			(17)				17.5	18	20
26½						(16)			

* The times enclosed in brackets refer to individual cases and are not averages.

The telophase in *Triton* erythrocytes is reported to take 50 per cent of the total time of mitosis, and in protozoa 53.5 and 60 per cent. Lewis and Lewis state that the telophase "which can be more accurately recorded than the other phases, shows a striking similarity in all types of cells and much less variation." If we take their telophase and reconstruction periods together as corresponding to the definition of the telophase used here, we find that the telophase in cultures of chicken mesenchyme and smooth muscles lasts about 50 per cent of the whole time of division, while the telophase of the zebra fish blastomeres takes only about half of this relative time, that is, 26 per cent. Even if the interkinetic phase, the delimitation of which is not at all clear, is added quite arbitrarily to the telophase, there is not more than 33.5 per cent of duration time accounted for. The certainty with which this result is obtained leads to the conclusion that the relative shortness of the telophase is actually significant for the type of karyokinesis we are dealing with, which involves the formation of

chromosomal vesicles in the telophase, and has no actual "resting phase."

The effect of different temperatures on the duration of cleavage is shown in Table III. The results cover only a part of the large range of temperature which the eggs can stand. The only conclusions, therefore, which can be safely drawn are that the duration of cleavage divisions is influenced by even slight changes in temperature, but that the general trend of acceleration for the first six cleavages, and the following retardation, are practically unchanged so that the duration of mitosis may be said to be constant under constant conditions. Many investigators, however, have found the duration of mitosis varying up to several hundred per cent for the same kind of cells. Observations of cells in tissue cultures in particular have yielded results which were very inconsistent with respect to the total duration of division. In all these cases the inconsistency can be attributed only to varying conditions of nutrition, oxygen supply, and temperature. A comparison of the results given in the literature and the observations on the eggs of the zebra fish, show that under constant conditions the duration of mitosis is constant and characteristic for the different types of cells.

Discussion

The process of cleavage is characterized by continuous and often synchronous cell divisions, which frequently follow a definite pattern. In general there is no morphological differentiation during cleavage, but very often there is a segregation of different materials in different cells. At the end of the cleavage period there is a break in the development, the divisions cease to be continuous and synchronous, and the period of cell migration and arrangement begins, often together with the first histological differentiation. On the other hand, cleavage is regarded as "but a continuation . . . of that series of cell-divisions which has been going on uninterruptedly, though with periodic pauses, since the most remote antiquity. The divisions of the egg during cleavage are in all essentials of the same type as those of adult cells; such differences as may appear—e.g., the prominence of asters, the frequent asymmetry of the amphiaster, and the consequent inequality of cleavage—are of minor importance, though often interesting for analyzing the mechanism of mitosis." (E. B. Wilson, 1928, page 981.) The general conception is that cleavage divisions are dynamically somewhat different from the divisions in the older animal, but that their variation is not correlated with any essentially different mechanism. There are, however, observations which point to a difference in mechanism. Investigators of the chromosomal vesicles, which so

frequently occur in the telophase of cleavage divisions, have often suspected that this particular feature of mitosis might be immediately connected with the fact that cleavage divisions go on continuously and almost without interphases.

The study of karyokinesis in zebra fish blastomeres reveals that the formation of chromosomal vesicles is obviously in itself a process of much shorter duration than the common type of telophase and, furthermore, that it represents a condition which permits of an almost immediate start of the next division without a "resting stage" and without a spireme formation in the prophase. No nucleoli are formed in this type of mitosis. All these features are characteristic only for the divisions during cleavage. About the time of the twelfth cleavage an entirely different type of mitosis appears, which shows no chromosomal vesicles in the telophase, but nucleoli and a very distinct spireme in the prophase (Fig. 5). In my material no transitional forms have been observed between these two types, though it is quite possible that a more thorough investigation may reveal such transitions.

Chromosomal vesicles have been found in the eggs of very many species and almost all classes of animals with the possible exception of birds and mammals. (A review of the literature has been given by Richards, 1917.) The suggestion seems obvious that the type of mitosis which is characterized most strikingly by the formation of chromosomal vesicles in the telophase, is due to some aspect of the division mechanism that is peculiar to the cleavage divisions. We have not yet arrived, however, at any definite conclusions concerning the possibly different dynamics involved.

Summary

The nuclei in the blastomeres of *Brachydanio rerio* can be observed easily in life. They are visible in the prophase and telophase as well as in the interkinetic phase. This discovery is used (1) to confirm and consolidate the results obtained from sectioned material; (2) to fix the blastomeres in any desired mitotic phase; and (3) to determine the duration of mitosis and its phases.

The duration of mitosis and its phases under constant conditions, particularly with respect to temperature, is found to be constant for each cleavage. The time from the breakdown of 32 nuclei to the breakdown of 64 nuclei is 18 minutes at 25° C. This places the cleavage divisions of the zebra fish among the most rapid ever observed. The first six cleavages show a trend towards acceleration, the sixth being the most rapid one. From then on the speed of the divisions slows down. This trend is essentially undisturbed by changes in temperature.

The nuclear divisions during cleavage are characterized (1) by the formation of chromosomal vesicles in the telophase (some of these vesicles can frequently be seen in life to persist through the interphase); (2) by a comparatively short duration of this type of telophase; (3) by a very short, if any, true interphase; (4) by the lack of nucleoli; and (5) by the absence of a typical spireme formation in the prophase.

The very short duration of the telophase has been recorded for the first time. The other observations have been found in the cleavage divisions of a majority of the species examined. In the zebra fish egg they continue until about the twelfth cleavage, when the form of mitosis typical for the adult first appears. It is suggested that this type of mitosis is probably associated with the rapid sequence of divisions and is generally characteristic of cleavage mitoses. The most characteristic feature of this type of karyokinesis is the formation of the chromosomal vesicles, but the shortening of the interphase and telophase, and the lack of spireme formation in the prophase are also obvious.

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THE EFFECTS OF LIGHT AND TEMPERATURE ON THE MALE SEXUAL CYCLE IN FUNDULUS

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Fundulus heteroclitus is a teleost fish which breeds during the late spring and early summer months. Its gonads undergo fairly definite seasonal changes, reaching their greatest weight just before the breeding season, falling off sharply just after spawning is completed in late July, then undergoing a period of slow growth until the onset of rapid maturation of germ cells prior to the next breeding season (Matthews, 1938). Several factors, acting independently or collectively, may be concerned in the control of this gonad cycle. Of these factors the pituitary gland as an internal factor and temperature and light among the external factors might reasonably be supposed to possess some degree of control. Evidence concerning the rôle that the pituitary gland plays in the sexual cycle has already been obtained (Matthews, 1939). The following experiments are concerned with the effects of light and temperature on the male sexual cycle.

From the experiments of a number of workers, particularly those of Bissonnette (see his review article, 1936) it is clear that in some birds and mammals light plays a dominant rôle and temperature a subordinate one in controlling the seasonal cycle in the gonads. The data on poikilothermous animals are not as conclusive. Clausen and Poris (1937) in the case of *Anolis* and Burger (1937) in the case of *Pseudemys* both believe that light is important in controlling the sexual cycle. Bellerby (1938), on the other hand, finds no evidence "that light is essential for the maintenance of reproductive activity in *Xenopus laevis* or that seasonal variation in light intensity or wave length plays any part in the control of the sexual cycle under natural conditions." Turner (1919), in describing seasonal changes in the spermary of the perch, pointed out that tremendous synthesis of material in the testis occurs in August when the temperature of the water has reached its peak and begun to decline, and expulsion of the sperm occurs when the temperature begins to rise. No experiments controlling the light factor were described. From his work with the stickleback Craig-Bennitt (1930) also concludes that temperature is the important factor in controlling the sexual cycle and that light is

unimportant. More recently Hoover and Hubbard (1937) have shown that a gradual increase in daily illumination followed by a gradual decrease will cause brook trout (a fall breeding animal) to produce ripe eggs and mature sperm several months earlier than normal.

To determine whether or not the absence of light exerts any inhibitory influence on spermatogenesis in *Fundulus* the following two experiments were carried out. In the first, begun in December, 11 males were divided in such a way that 5 were maintained in an aquarium subjected to ordinary daylight with no night illumination and the other 6 were kept in a light-proof tank. They were fed daily with the stock food used in all experiments, consisting of dried shrimp, puppy biscuit and Mead's infant cereal, with occasional living food such as worms or *Daphnia*. These animals were killed at five different intervals over a period of 3 weeks. The average percentage of the body weight formed by the testis of the animals in the light-proof tank was 0.56 per cent, that in the other aquarium 0.37 per cent, and microscopic examination of the testes showed no significant differences in the state of activity of the two groups. In the second experiment, begun in March and extending into April, 4 males were placed in the lighted aquarium, which in this instance was illuminated at night by a 50-watt mazda bulb suspended above the tank, and 8 were kept in the dark. The animals were killed over a period of 4 weeks. The percentage of the body weight formed by the testis in the illuminated aquarium averaged 1.76 per cent, in the darkened tank 1.88 per cent, and again no structural differences were observed in the microscopic structure of the two groups of testes. In these cases some of the animals killed during April presented the white testis and numerous sperm associated with a high degree of activity, and this occurred as early in the darkened tank as in the illuminated one. From these experiments it seems fairly clear that absence of light for at least 4 weeks prior to the breeding season does not inhibit activation of the testis in *Fundulus*. It should, of course, be noted that the animals which developed ripe testes in the dark had been on a rising daylight curve for nearly three months before they were placed in the dark. Whether or not this is a significant factor in initiating the active phase in the testis cycle has not been determined.

Experiments concerning the effect of temperature on activation of the testis gave somewhat different results. Ten animals were kept in a tank in which the temperature of the water averaged 21° C. (variation 19°–21.5°) and 10 animals were kept in a constant temperature room with light conditions similar to those of the first group, the temperature of the water in the aquarium here averaging 5.5° C. (variation 4°–7° C.).

In a series run during December the testis formed as large a percentage of the body weight of animals in the cold room as in the normals (0.53 per cent), but sections of the testes of animals after 23 days in the cold room showed that these were retarded in development, particularly in the later stages of spermatogenesis. In a series run during March and April, moreover, the testes of those maintained at 5.5° C. averaged only 1.16 per cent of the body weight as against 1.76 per cent for those in the warmer room and in general animals maintained in the cold produced sperm much later than did those in the warm room. The retarding effect of the low temperature was noted in this series as early as 9 days after the beginning of the experiment. Only one individual, killed April 5 after 21 days in the lower temperature, showed a degree of activity comparable with that of the control animals.

In brief, then, records have been obtained on 14 animals maintained in a light-proof tank from 4 to 55 days as compared with controls subjected to daylight or to daylight and added night illumination. The testes of these animals were like those of the controls and in cases killed late in April those of both groups were whitish and filled with sperm. Only one case, killed December 26 after 23 days in the dark, showed a testis less developed than that of the control. Records have also been obtained on 17 animals which were maintained at a temperature of 5.5° C. as compared with 13 control animals kept at 21° C. After 9 to 23 days at the lower temperature spermatogenesis was definitely retarded.

These experiments show that the presence of light is not essential for complete activation of the testis of *Fundulus;* and that low temperatures exert a retarding influence on maturation of the sperm. Obviously no evidence is furnished concerning the effects of gradual changes in the amount of light to which the animal is subjected daily, which Hoover and Hubbard found of such importance in the sexual cycle of the trout.

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SOME EXPERIMENTS ON THE RELATION OF THE EXTERNAL ENVIRONMENT TO THE SPERMATOGENETIC CYCLE OF FUNDULUS HETEROCLITUS (L.) [1]

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INTRODUCTION

Within the last decade a considerable body of experimental work has shown that the sexual cycles of many vertebrates of the north temperate zone are regulated in part by the annual cycle of changes in day-length. Little, however, is known about the relation of the external environment to the sexual cycle of cold water fish. Success in modifying the piscine sexual cycle by light agencies has been reported for the trout (Hoover, 1937; Hoover and Hubbard, 1937), for the minnow, *Phoxinus* (Spaul cited from Rowan, 1938), and for the stickleback (Tinbergen cited from Rowan, 1938). Craig-Bennett (1930) came to the conclusion that the sexual cycle of the stickleback was regulated primarily by temperature. Hoover (private communication to T. H. Bissonnette) has found that light is ineffective on yellow perch which were kept in water below 44° F.

The normal sexual cycle of *Fundulus* has been described by Matthews (1938). As in many cold-blooded vertebrates the sexual cycle is a continuous process throughout, with no genuinely inactive phase, although during the winter there is little or no spermatogenetic activity. In the late summer and fall a limited production of spermatogonia takes place. Vigorous spermiogenesis begins in the spring, with a mating period during May and June. Thereafter occurs a gradual deceleration of spermiogenesis with a concomitant testicular involution.

It is noticed that the major portion of the spermatogenetic activity is present during the spring when the days are increasing in length, and when the temperature of the water is rising. The experiments here reported are to test the relation of light and temperature to the spermatogenetic cycle of *Fundulus*.

[1] Aided in part by a grant from the American Philosophical Society administered by T. H. Bissonnette for 1938–39.

Materials and Methods

Over seven hundred newly captured adult male *Fundulus* were used in four experiments. Two of these experiments were performed in Maine, and two in Connecticut. Fish were secured on June 30 from a tidal inlet on Mt. Desert Island, Maine, and were confined to laboratory aquaria fed by sea water. One control aquarium was placed out-of-doors in a well lighted spot. The fish therein were maintained on natural daylight until August 27. Two other aquaria were placed in a light-proof box, which was illuminated by two 50-watt lamps. From June 30 to July 22 the daily light ration was reduced 20 minutes per day from 15 hours to 8 hours. Then between July 22 and August 27 the daily light ration was increased 20 minutes per day from 8 hours to 20½ hours. The temperature of the aquaria water ranged between 11° and 17° C., in general increasing in warmth from June to August.

On October 29 *Fundulus* were secured from a tidal inlet off Long Island Sound near Niantic, Connecticut. These animals were placed in fresh water aquaria at Hartford, Connecticut. The control fish were exposed to daylight for natural day-lengths between October 29 and January 4. The experimental fish received in addition to natural daylight illumination from a 100-watt lamp. The exposure to electric light was increased every 5 days so that by December 11 the fish were receiving 8½ hours of electric illumination added to normal daylight. After this date no further increases in the length of time of exposure to light were made. The temperature of the fresh water aquaria ranged between 11° and 18° C., decreasing in warmth from October to January.

In a final experiment fish were captured from a tidal inlet at Old Lyme, Connecticut, on February 25, and were confined to fresh water aquaria at Hartford. All aquaria were made light-proof. The fish never received more than 1½ hours of light per day during the experimental period which was from February 25 to March 25. This limited exposure to light was necessary for feeding. One group of males were kept in cold water which varied in temperature between 6° and 10° C. Another group was kept in warmer water which ranged between 14° and 20° C. There was always at least 6° C. difference in temperature between the two groups.

Fish were also sampled from the wild at various intervals. The animals were fed almost daily on either chopped livers or clams. Only healthy, fungus-free fish were killed for histological study.

Results

Confinement to aquaria and to fresh water had no deleterious effects on the testes. Judging from the condition of the internal organs, the diet was more than adequate for the maintenance of good health.

In all the experiments with light, and where there was no significant difference in temperature between controls and experimentals, no difference in the state of the testes was found between control and experimental fish. The experimental light rations were: 21 days of gradually decreased lighting between June 30 and July 22; 37 days of gradually increased lighting between July 22 and August 27; and 68 days of gradually increased lighting between October 29 and January 4.

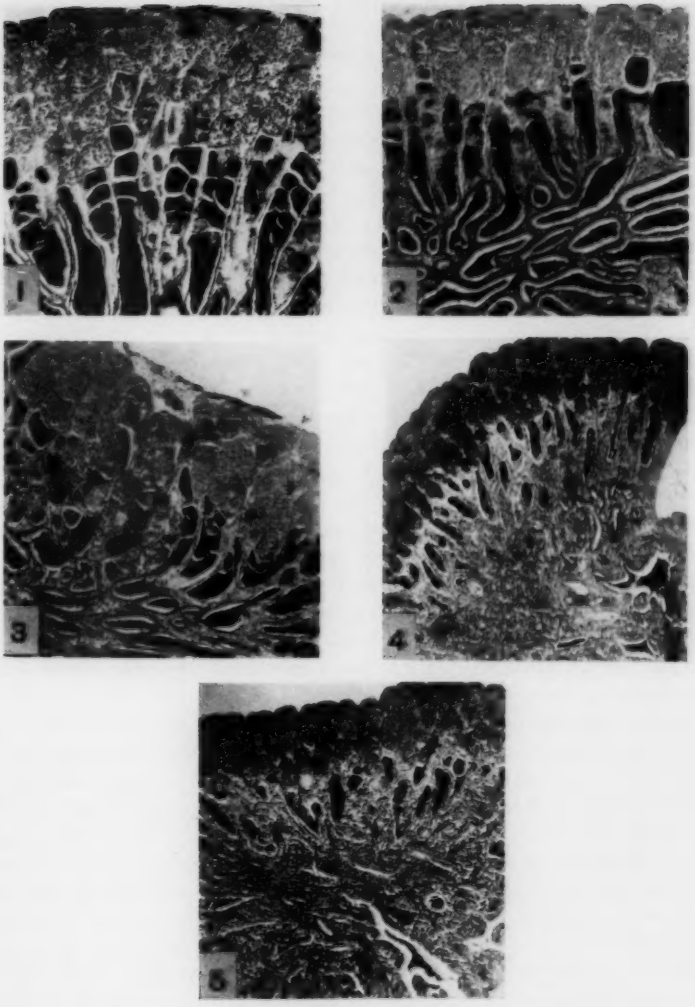
On June 30 at the start of the first experiment, spermiogenesis was at its peak. As can be seen in Fig. 1, there exists at this time a broad zone of cortically located spermatogonia etc. surrounding medullary tubules which are filled with sperm. During the summer there occurs a gradual decrease in the proliferation of spermatogonia, together with a loss of sperm in the tubules. The rate of this testicular involution can be seen by comparing Figs. 1, 2, and 4. Figures 2 and 4 are from laboratory control fish for July 22 and August 27 respectively.

That the fish which received 21 days of gradually decreased lighting showed no differences in testicular state when compared with control fish, is illustrated in Figs. 2 and 3. Figure 2 is from a control testis, and Fig. 3 from an experimental fish whose light ration was gradually reduced to 8 hours per day. Likewise a comparison of Figs. 4 and 5 shows that there was no difference in testicular state between control fish (Fig. 4) and experimental fish (Fig. 5) after 37 days of gradually increased lighting applied to *Fundulus* which previously experienced 21 days of decreased lighting.

These results indicate that the testicular involution which normally occurs during the summer when the day-lengths are naturally shortened cannot be hastened by 21 days of decreased lighting. Moreover, 37 days of subsequent increased lighting does not change the rate of testicular involution, nor does it induce a precocious new spermatogenetic cycle. Since many animals are refractory to photoperiodic manipulations at the end of their sexual cycle, the above experiments are no test for the efficacy of photoperiodic manipulations on the sexual cycle.

The fish which were lighted for 68 days between October 29 and January 4 offer a fair test, however, as to whether or not the sexual cycle of *Fundulus* can be influenced by light. When this experiment was begun, the cortical zone of the testis was slowly proliferating spermatogonia, while the medullary system of tubules was involuted and devoid

PLATE I



All figures are unretouched photomicrographs, × 80.

FIG. 1. Section of a testis from a fish captured 6/30/38. The cortical zone (at the top of the figure) of spermatogonia, etc. is broad; the medullary zone of tubules is black with sperm.

FIG. 2. Section of a testis from a laboratory control, 7/22/38. The spermatogonia are fewer than in Fig. 1, and the tubules contain fewer sperms.

FIG. 3. Section of a testis, 7/22/38 after 21 days of shortened day-lengths. The testis is in the same condition as that of the controls (Fig. 2).

FIG. 4. Section of a testis from a laboratory control, 8/27/38. Spermiogenesis is almost finished; the tubules have markedly involuted.

FIG. 5. Section of a testis, 8/27/38 after 37 days of increased lighting. No significant difference is found between this and the control (Fig. 4).

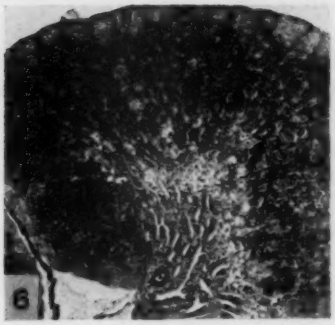
of sperm. This condition can be seen in Fig. 6, which is a section of a testis on October 29.

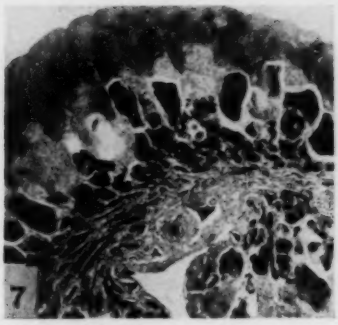
During this experiment sperm were produced both by the control and experimental fish. These sperm can be seen in Fig. 7 which is from a control fish on January 4, and in Fig. 8 which is from an experimentally lighted fish on January 4. In the control fish, sperm were formed while the days were decreasing in length, as indicated by fish sampled in December. In the experimental fish, sperm were formed no more abundantly when the day-lengths were increased in length by means of 8½ hours of electric light added at night. Thus for fish at the threshold of a new spermatogenetic cycle, the application of increased or decreased daily rations of light does not modify the rate of the subsequent formation of sperm.

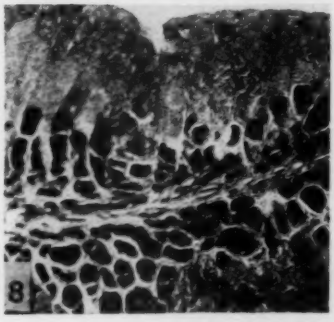
A comparison of laboratory fish and fish from nature in early January showed that the fish in their natural habitat do not form sperm at this time as did the laboratory fish. This statement needs to be qualified slightly for there are *Fundulus* in nature which during the winter form a very few spermatozoa. However, the general condition for winter fish at least up until early March is similar to that shown in Fig. 6. The most obvious difference between the laboratory fish and the fish from nature is the difference in water temperatures. The laboratory fish lived in water between 11° and 18° C., while the fish in nature during the winter lived in water whose temperature was near 0° C.

The experiment with temperature where the daily light ration was only long enough for feeding the fish indicates that spermatogenesis is responsive to temperature manipulations. Figure 9 is a section from a testis from a fish after 29 days in water whose temperature varied between 6° and 10° C. These temperatures were somewhat higher than those experienced by the fish in nature at the time of capture and during the experimental period. This testis is a winter testis and shows no transformations of spermatozoa. However, there did occur a slight multiplication of spermatogonia so that the testis was not completely inactive during this period. Figure 10 is a section of a testis from a fish after the same 29 days in water whose temperature varied between 14° and 20° C. Here spermatozoa have been formed in large numbers. This effect of higher temperature can readily be seen by comparing Figs. 9 and 10. This result was uniform for all fish. It should be emphasized that these fish never had more than 1½ hours of light per day during the experimental period, and usually not more than one-half hour.

PLATE II







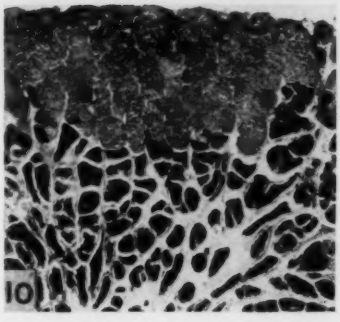


FIG. 6. Section of a testis from a fish captured 10/29/38. The bulk of the testis consists of spermatogonia. Sperms are absent and the tubular system is greatly reduced.

FIG. 7. A section from a laboratory control, 1/4/39. The black areas are tubules which contain sperm.

FIG. 8. A section of a testis, 1/4/39 after 68 days of increased lighting. Sperms while abundant are no more numerous than in the controls (Fig. 7).

FIG. 9. A section of a testis, 3/25/39 after 29 days in almost complete darkness in water whose temperature varied between 6° and 10° C. This is essentially a winter testis consisting of spermatogonia (compare with Fig. 6). Sperms are absent.

FIG. 10. A section of a testis, 3/25/39 after 29 days in almost complete darkness in water whose temperature varied between 14° and 20° C. The black areas show the large numbers of sperms that have formed.

Discussion

These experiments indicate that light as such is of no importance in the spermatogenesis of *Fundulus*. Spermatozoa can be formed in almost complete darkness, and will form in equal abundance when the days are either increasing or decreasing in length. Temperature appears as the important factor of the external environment which modifies spermatogenesis. In cold water spermatogenesis is retarded or inhibited, while in warm water spermatogenesis is rapidly completed.

These experiments give no exact data on the critical temperatures involved, but from our observations both on experimental fish and on fish in nature a general scheme seems clear. At temperatures near 0° C. the testis is inactivated. As the temperature rises toward or around 10° C. spermatogonial multiplications occur. Still higher temperatures permit the transformations of sperm to take place.

Marine temperatures show a very orderly annual cycle. Dr. R. A. Goffin kindly gave us the mean daily sea water temperatures for 1938 at Woods Hole, Massachusetts. Dr. V. L. Loosanoff also referred us to his paper (Loosanoff, 1937) which gives the shallow water temperatures for over three years at Charles Island, Long Island Sound. Both sets of data show a low point in February followed by a continued rise beginning in March and reaching a maximum in August. From August to September a continued drop takes place.

The spermatogenetic cycle of *Fundulus* fits nicely into this annual temperature curve. In the fall as the temperature drops spermatogonial multiplications take place. During the winter the testis is relatively inactive. In fact, fish captured in late February show slightly less testicular activity than those captured in early January. The spring rise in water temperature is accompanied by increased spermatogenetic activity. It should be remembered that in nature the beginning of active spermatogenesis coincides with the warming of the water, and not with the increased lengthening of the days which began three months previously.

Summary and Conclusions

1. No differences in the velocity of the spermatogenetic cycle of adult male *Fundulus* were found between control and experimental fish kept in water of the same temperature when treated as follows: (*a*) 21 days of gradually decreased day-lengths between June 30 and July 22, (*b*) 37 days of gradually increased lighting subsequent to treatment as in (*a*) between July 22 and August 27, (*c*) 68 days of increased lighting between October 29 and January 4.

2. Laboratory fish kept during the late fall and early winter in

water whose temperature was higher than that experienced by fish in nature showed an acceleration of spermatogenesis.

3. Laboratory fish which received no more than 1½ hours of light per day and which were kept in water of from 6° to 10° C. between February 25 and March 25 remained inactive sexually. Fish which received no more than 1½ hours of light per day and which were kept in water whose temperature varied between 14° and 20° C. formed large numbers of sperm within this same period of time.

4. It is concluded: (*a*) that the spermatogenetic stages of the annual sexual cycle are not affected by light as light; (*b*) that the temperature of the water is the important factor of the external environment regulating spermatogenesis in *Fundulus*.

5. It is suggested that at temperatures near 0° C. sexual activity is inhibited. As the temperature rises toward or near 10° C. spermatogonial multiplications occur. Still higher temperatures produce complete spermatogenesis.

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INFLUENCE OF THE SINUSGLAND OF CRUSTACEANS ON NORMAL VIABILITY AND ECDYSIS[1]

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Since the work of Perkins and of Koller in 1928, who independently described the presence of a substance in eyestalk extract which exercises a very potent effect upon the chromatophores of crustaceans, there has been much interest shown in the crustacean eyestalk function. The picture has been rendered even more interesting as a result of the work of Brown (1935) and of Kleinholz (1938), demonstrating that humoral activity in this group of animals is by no means a simple one, but that several hormonal substances are normally functioning. Hanström (1935) performed experiments in which he showed that the portion of the eyestalk which was active in affecting chromatophores always contained, among other things, a tissue which he has termed the sinusgland. This has given rather good evidence indicating which tissue of the eyestalk is the active one in this regard. The cells of this tissue were shown to be secretory in nature and to contain a rich supply of secretory granules. The more recent work of Hanström (1936), Stahl (1938) and others have shown the sinusgland to be present in some degree in all the crustaceans that have been examined in detail. Its occurrence appears to be quite independent of the state of development of a chromatophore system. Functionally it appears to have common properties with the corpora allata of insects since an extract of the latter organ in many cases serves as an activator of crustacean chromatophores. Abramowitz (1936, 1938) has demonstrated that the chromatophorotropic substance from the sinusgland and the intermedin of the vertebrates have certain common chemical and physiological properties.

Koller (1930) was the first to demonstrate that the eyestalk substance has another function in addition to the control of chromatophores. He found that animals from which the eyestalks had been removed failed to deposit calcium in their exoskeletons to the same extent as normal animals. He interpreted this to be the result of removal of the source of a controlling hormone. Welsh (1937) found that when he perfused an exposed crayfish heart with eyestalk extract

[1] This investigation was supported by a research grant from the Graduate School of Northwestern University.

there was a pronounced speeding up of that organ. Brown (1938) demonstrated that removal of the eyestalks appreciably shortened the life of the individual and that the shortening thus induced could be compensated for in part by implantation of eyestalk tissue into the ventral abdomen. This shortening of the life of the animal has been called a "viability effect" of an eyestalk hormone, though it is fully realized that this is a function described in far too general terms. It is hoped that this "viability effect" can soon be analyzed into the particular phenomena responsible for the shorter life.

There has frequently been suggestion of a "molting effect" of the eyestalk substances, though no adequate data have yet been published to establish such a function. The only grounds for such a belief are that several investigators have mentioned that eyestalkless animals appear to molt more frequently than normal ones. No reason has been advanced for thinking the effect is due to anything other than the injury caused by the operation of eyestalk removal (indicated by Darby, 1938).

The following research has been conducted in continuation of that of Brown (1938) with the intention of discovering just what tissue of the eyestalk is responsible for the "viability effect" of this organ. There is included here the first direct evidence for an endocrine activity of the sinusgland of the crustacean. Hitherto its functioning had been supposed upon the grounds of the best of circumstantial evidence. During these experiments the sinusgland has been dissected out and implanted into the ventral abdominal sinus of eyestalkless animals. Direct physiological evidence of its endocrine function has been demonstrated. Furthermore, it is quite well established as a result of these experiments that this gland is the one responsible for the normal continuation of life of the animal and also that it has a functional activity in the control of molting. The possibility of explaining the viability effect of eyestalk hormones in terms of molt control will be discussed.

Methods and Materials

All the crayfishes used in these experiments were small individuals (carapace lengths 15–30 mm.) of the species *Cambarus immunis*, with the exception of certain large individuals (*Cambarus virilis*, *C. blandingii*, and *C. immunis* of carapace lengths 30–40 mm.) which were used as the source of the sinusgland for implantation. The animals were brought into the laboratory a few days before the beginning of an experiment. It was our purpose to use experimental extirpation and implantation to determine the normal functions of the eyestalk gland within the body.

The method of extirpation was simple: the eyestalks were removed as a whole and the wound sealed with an electric cautery. By so sealing the wound, less than 10 per cent of the animals died as a result of the operation. It is fully realized that such a method of gland extirpation removed much tissue in addition to that of the sinusgland.

In the first experiment to be described the implantation consisted of all the eyestalk tissue. The eyestalks were removed from an animal and dropped into amphibian Ringer's solution. With the aid of a dissecting microscope the exoskeleton of the eye was cut away. The soft parts of the eyestalk were easily removed with fine forceps. This tissue was then teased into minute fragments and injected by means of a glass capillary pipette into the ventral sinus of the abdomen. The glass pipette proved to be especially satisfactory since it was possible to ascertain that all of the tissue entered the animal and none was left adhering to the walls of the pipette.

In those experiments in which the sinusgland by itself was to be implanted the gland was carefully dissected out in the following manner: the eyestalk was removed from a large crayfish and dropped into a watchglass containing amphibian Ringer's solution or a balanced salt solution based on Griffeths' analysis of *Astacus* blood (which will henceforth be referred to as Griffeths' solution). With a pair of sharp pointed scissors the chitinous exoskeleton was clipped to free the dorsal half of the stalk skeleton from the ventral half. The contents of the stalk were then picked out with fine pointed watch-maker's forceps and the dorsal tissue was teased away in the direct light of a strong lamp. The sinusgland tissue stood out quite conspicuously as a seemingly fibrous and granular bluish tissue. This mass of tissue was easily torn away from the adjacent nerve tissue. All the adhering tissue was teased away and the gland rinsed in amphibian Ringer's or Griffeths' solution. With forceps the gland was next pushed through an opening made in the ventral side of the abdomen. The clear exoskeleton in this region made it possible to ascertain that the minute gland was actually left in place upon removal of the forceps.

In order to determine the exact location of the tissue removed from the eyestalk, sections were made of the bluish gland-like tissue that was removed, and also of all the remaining portions of the eyestalk. In addition, longitudinal sagittal sections of the complete eyestalk were made as a control. By study of these three sets of sections it was readily determined just what tissue was being implanted. It was discovered that the implant tissue in histological section appeared to be definitely glandular in nature and occupied a position wedged between the medulla externa and the medulla interna. Con-

sidering its position and the fact that its cytoplasm was richly charged with eosinophilic inclusions, it seemed highly probable that this gland was the same as that described by Hanström (1936) as the sinusgland. The accompanying photographs show this gland as it occurs in *Cambarus virilis*. The first photograph is a median sagittal section of the

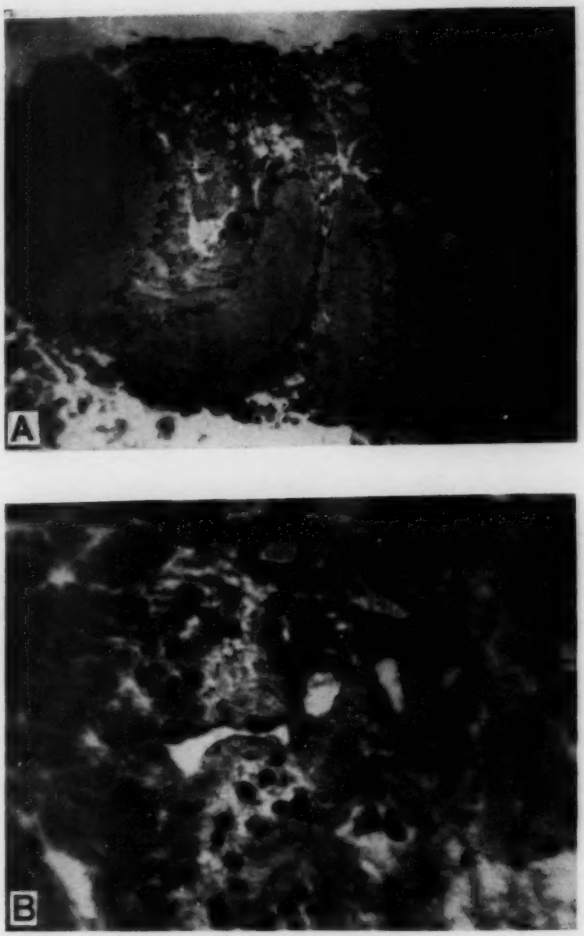

FIG. 1. Sagittal sections through the eyestalk of *Cambarus virilis* (6 micra thick and stained with Delafield's haematoxylin and eosin). *A*. At a magnification of 80 ×, showing the sinusgland as a somewhat triangular section of tissue located dorsally to a point intermediate between the medulla externa and the medulla interna. *B*. A higher magnification (360 ×) of the central region of the sinusgland.

eyestalk at a magnification of approximately 80× and the second is a higher power magnification (about 360×) in the central region of the gland.

During the experimental period all the animals were kept in individual glass finger bowls in water not quite deep enough to cover the carapace. These finger bowls were covered loosely with glass plates to minimize evaporation of the water but still to permit circulation of air over the water surface.

The experiments performed included extirpation and implantation, with appropriate controls, and observations were made upon viability and molt behavior.

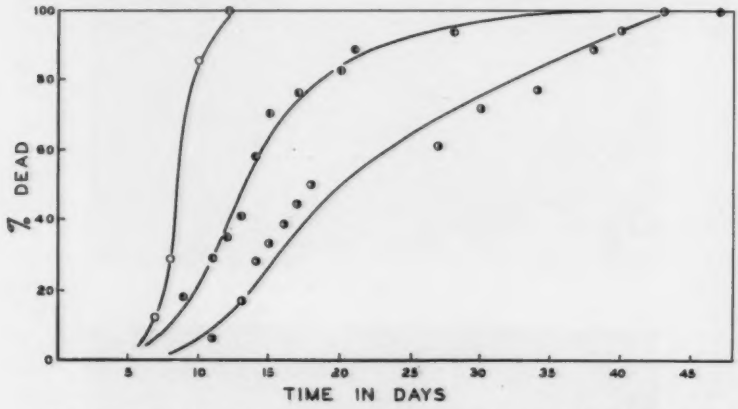

FIG. 2. The relation between the percentage of animals dead and the number of post-operative days for eyestalkless crayfishes, (○); eyestalkless crayfishes with a heteroplastic implant of sinusglandless eyestalk tissue, (◐); and eyestalkless crayfishes with only a heteroplastically implanted sinusgland, (◑).

EXPERIMENTS ON VIABILITY EFFECTS

Experiment I

The animals of this experiment, all *Cambarus immunis* with both eyestalks removed and the stubs cauterized, were divided into three lots. In the first lot were 7 animals with no further treatment. The second lot of 17 animals had a sinusgland taken from a single eyestalk of a large *Cambarus virilis* or *Cambarus blandingii acutus* implanted into the ventral sinus of their abdomens. The third lot of 18 animals had an abdominal implantation consisting of all the eyestalk tissue of a single eyestalk of *Cambarus virilis* or *Cambarus blandingii acutus*, from which the gland had been carefully removed.

The results of this experiment are best shown in the form of a graph (Fig. 2) in which the percentage of animals dead is plotted

against the post-operational day. This graph demonstrates clearly that eyestalkless animals without abdominal implants live significantly shorter lengths of time than eyestalkless animals into which eyestalk tissue *minus the sinusgland* has been implanted. Similarly, eyestalkless animals which have received abdominal implants of the minute sinusgland by itself, live very significantly longer than those animals into which the remaining portion of the eyestalk tissue was implanted. Comparing only the instance of sinusgland implant with the case of no implant, we can conclude definitely that the minute sinusgland lengthens the post-operative life of the animal considerably. It is well to bear in mind that these two latter groups have been subjected to operations of different degrees of severity, in which the animals which

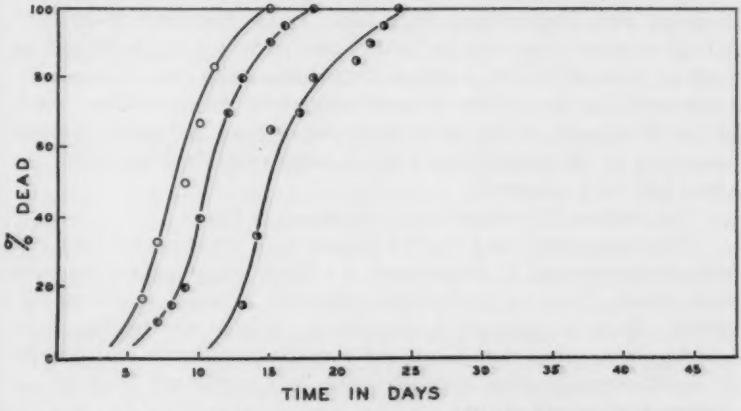

FIG. 3. The relation between the percentage of animals dead and the number of post-operative days for eyestalkless crayfishes, (○); eyestalkless crayfishes with a homoplastic implant of sinusglandless eyestalk tissue, (◐); and eyestalkless crayfishes with only a homoplastically implanted sinusgland, (◑).

live longer have been subjected to more severe operative injury, the animals of the latter group having their abdomens punctured as well as having both eyes removed. A logical explanation of the intermediate length of post-operative life in the instance of those animals with the glandless stalk tissue implants is that there is present in the blood spaces of the general eyestalk tissue a product that has arisen from the sinusgland. During its removal, a bluish liquid is seen to diffuse out of the gland and infiltrate into the surrounding tissues. The general stalk tissue is frequently filled with a homogeneous blue liquid which, in all probability, comes from the same origin. We believe, therefore, that this additional substance is responsible for permitting these animals to live longer than those in which no implant

is made. It is also possible that fragments of the gland itself still remain which were not removed at the time of operation.

The implants in this experiment are heteroplastic, while in an experiment to be described later all the implantations were autoplastic as was the case with those observations published by Brown (1938). It becomes doubly interesting that the sinusgland has a definite effect not only upon the length of post-operative life in the same species of animal, but that the tissue from one species is capable of working effectively within the body of another species to the same end. Thus these substances, or this substance, is inter-specifically active.

Experiment II

In this experiment, like the preceding one, eyestalkless *Cambarus immunis* were divided into three lots. In the first lot, consisting of 6 large animals, there was no further treatment. A single sinusgland from an eyestalk of a large animal of the same species was abdominally implanted into each of the 20 small animals of the second lot. Each of the 20 animals of the third group received an abdominal implant consisting of the tissue from a single large eyestalk from which the gland had been removed.

The results of this experiment are shown in Fig. 2.

This experiment confirms the influence of the sinusgland on viability demonstrated in Experiment I. Here the implantations were homoplastic, from large *Cambarus immunis* to small *Cambarus immunis*. As in Experiment I, the animals without any implant lived a much shorter time than those with sinusgland implants, and animals in which sinusglandless eyestalk tissue was implanted lived for an intermediate length of time.

Experiments on the Molting Control Function of the Sinusgland

Experiment I

This experiment was intended to discover any differences that might occur in the molting process among animals from which both sinusglands had been removed, one sinusgland removed, both sinusglands removed but with them autoplastically implanted into the ventral abdominal sinus, and finally, completely normal animals.

In this experiment four lots of animals were isolated. The first lot of 34 animals was left in perfectly normal condition, though placed in the usual individual glass finger bowls with covers. The second lot of 48 animals was subjected to removal of one eye each. A third lot of 79 animals had both eyestalks removed in the usual manner. The

fourth lot of 44 animals had both eyestalks removed and the contents of their own eyestalks in amphibian Ringer's solution injected into the ventral sinus of the abdomen. Observations were made only with regard to actual molting. The results that were obtained are summarized in Table I.

TABLE I

Data indicating the extent of molting in crayfishes under different experimental conditions.

	Normal Animals	One Eye Off	Two Eyes Off	Two Eyes Off (Implant)
Total no. examined....	34	48	79	44
No. "molts".....	9	19	23	3
Per cent "molts".....	26	40	29	7
Per cent "molts" dying in process.....	44	16	74	100
Per cent molt/av. life span.....	2.0	3.4	5.75	1.0

All the records of molting in Table I indicate instances in which the animal either completed the molt or was well along in the process at the time of death. The most significant portion of the table is the item "per cent molt/average life span" which gives the only true figure of the relative rates of molt. The "per cent molts" fail to do this inasmuch as the different lots of animals survived different lengths of time; consequently such animals as normal animals and those with one eyestalk off had a longer time in which molts could occur. On this strictly relative behavior (per cent molt/average life span) the figure for normal animals is 2. With one eye removed, the rate of molt is increased by about 75 per cent, and with the removal of two eyes the molting has been accelerated about 200 per cent. The striking fact, however, is that when both eyes were removed and the eyestalk tissue abdominally implanted, the figure indicating the molting rate is 1, or about half that of normal animals. Were it not for the anomalous molting rate of this last group the results could be interpreted as indicating that the rate of molting is a function of the extent of injury. But, taking the data together, there appears to be a more probable explanation. The eyestalk tissue, under nerve control, liberates a humoral substance into the blood which inhibits the molt. With one eye removed, relatively less substance is liberated and with two eyes removed none of the material, and we see molt correspondingly going on at relatively greater rates. In these terms the explanation of behavior of the last group of animals might be that the implanted glandular tissue continuously liberates some antimolting substance and the animal is almost unable to molt.

Some of the acceleration resulting after eyestalk removal may be due to injury effects, but that they are not totally due to injury is indicated by the implantation experiments.

Experiment II

This experiment points to the sinusgland in the eyestalk as the actual tissue involved in the formation of the molt control humoral substance. The data for this conclusion are taken from observations on molting in the animals in Experiment II on viability.

A consideration of the ratio of percentage of completed or nearly completed molts to average survival period, shows that the implantation of the sinusgland reduces the molting rate to about one-fifth of that which occurs in the controls with the glandless stalk tissue implants. The conclusions of the former experiment are confirmed and it is further indicated that the sinusgland is the effective tissue in molt control. The results of this experiment are summarized in Table II.

TABLE II

Data indicating the extent of molting in crayfishes under different experimental conditions.

	Two Eyes Off	Two Eyes Off (Implant)
Total no. examined	20	20
No. "molts"	4	1
Per cent molts	20	5
Per cent molts/av. life span	1.57	.31

In the course of this experiment all the animals were carefully watched, not only for completed molts but also for the slightest symptoms of the beginnings of molt. The early signs of molt were usually indicated by a visible separation between the carapace and the first abdominal tergite. Practically all of the eyestalkless animals, regardless of the type of implant, showed this separation from three hours to three or four days prior to their death. This was so definite that it was possible to predict the death of any animal within these limits. In many instances this separation was followed by a completed molt, though in the majority of cases the animals died before further steps in the molting process. It is admitted that some other factors, such as change in general tone of the abdominal musculature or upset in the water metabolism of the animal, might be operating in inducing the separation of these two skeletal elements. Superficially, however, we are unable to differentiate between the initiation of the normal molt and its induction by other causes. Furthermore, many of the animals showing this apparent initiation in the molt process showed

muscular activity of the body such as is usually associated with the normal molting process.

Those animals from which the eyestalks had been removed and which received the glandless eyestalk implantation, all showed the apparent initiation of molt or completed the molt prior to their death. In three cases the animals completed the molt before death, in one case dying within a day of the molt and in the other cases living two and four days, respectively, after molting. In a fourth case the animal died when well along in the molting process. These facts would indicate that even without the eyestalks the animals are physiologically able to complete the molt. But the fact that the eyestalkless animals sometimes continue to live several days after molting and then die without showing further signs of molt, indicates that the sinusgland has a function in addition to molt control.

The majority of the animals with sinusgland implants also showed the beginnings of molting prior to their death, just as did the first lot. The only difference between the lots seemed to be that the molting activity was postponed in the case of the implanted animals. These animals seldom do more than show this first sign of molt, scarcely ever proceeding far into the molt or completing it. A possible explanation of this is that these animals are prevented from molting by action of the implant until the absence of the eyestalk has worked other degenerating effects upon the organisms to the extent that they no longer have the power to go far with the molt, in spite of removal of the inhibitor through loss of function of the implant. In this regard it would be interesting to trace the rate of degeneration of the implanted tissue to see if there may be any correspondence between the time of oncome of the molt and the structural degeneration of the implanted cells.

It may be possible to interpret the data of Koller (1930) in terms of molt control activity. Animals molting more frequently as a result of absence of a hormone from the sinusgland might well be expected to have less calcium salts in their exoskeleton than normally.

Summary

1. Direct evidence for an endocrine activity of the crustacean sinusgland is given. This evidence has originated from implantation experiments.

2. Removal of the sinusgland significantly shortens the life of the animals, and conversely the length of life of animals with sinusglands removed can be significantly lengthened by implantation of the gland.

3. The sinusgland is readily dissected out in fresh eyestalk tissue

in strong reflected light. It has a distinctly bluish cast. It is a definite organ which can be readily teased away from the surrounding tissue and removed as a whole.

4. Certain evidence suggests very strongly that a substance concerned with the control of molting is elaborated in this gland. The most probable action of this substance is that of inhibiting molt.

5. The action of the sinusgland in molt control appears to be insufficient to explain the viability effect entirely.

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THE METHOD OF FEEDING OF CHAETOPTERUS

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Introduction

Ciliated currents present on the surface of animals, when examined under artificial conditions, are seldom, if ever, typical of the animal in its natural environment. Failure to recognize this fact and failure to observe the presence of mucus and note its importance in the feeding process have given rise to many erroneous descriptions of the feeding mechanism of various marine invertebrates. In conformity with the statement made in *Science* (MacGinitie, 1937), the feeding activities of many marine invertebrates have been investigated (including tunicates, pelecypods, gastropods, annelids and coelenterates), and descriptions of the feeding activities of these animals will follow as soon as they can be prepared for publication. This paper will deal with the feeding of the annelid *Chaetopterus variopedatus* Rénier et Claparède.

Because of its wide distribution and its usefulness as a source of embryological material, *Chaetopterus* is well known both abroad and in this country. Also, because of its unusual and somewhat bizarre structure, it has created a great deal of interest from both an anatomical and a natural history point of view (Laffuie, 1890; Enders, 1909). However, no paper that I have seen has given the correct method of feeding of this animal.

Feeding Method

The structures concerned with the feeding activities of *Chaetopterus* are the peristomial funnel with its lips, the mouth, the dorsal ciliated groove, which ends in the dorsal cupule of the thirteenth segment, the pair of aliform notopodia of the twelfth segment, and the three fans of the fourteenth, fifteenth and sixteenth segments (see Fig. 1).

In preparing to feed, *Chaetopterus* approaches one or the other end of the leathery U-shaped tube in which it lives and spreads its aliform notopodia out against the sides of the tube. It then begins to secrete mucus from the inner walls of these notopodia, the secretion beginning at the distal ends and proceeding inward toward the body. The cilia of the inner surface of the notopodia carry the mucus across the

opening in a sheet from the distal ends to the body of the worm, whence it is carried posteriorly as a bag by the ciliated groove to the dorsal cupule, where the closed end of the mucous bag is taken into the cup or concave surface of this organ. This creates an elongated bag of mucus, the anterior end of which is fastened to or continuous with the glands lining the inner surface of the aliform notopodia, and the closed posterior end of which is held by and rolled up within the dorsal cupule.

A current of water is now maintained through the burrow by the activity of the three fans just posterior to the dorsal cupule. Since

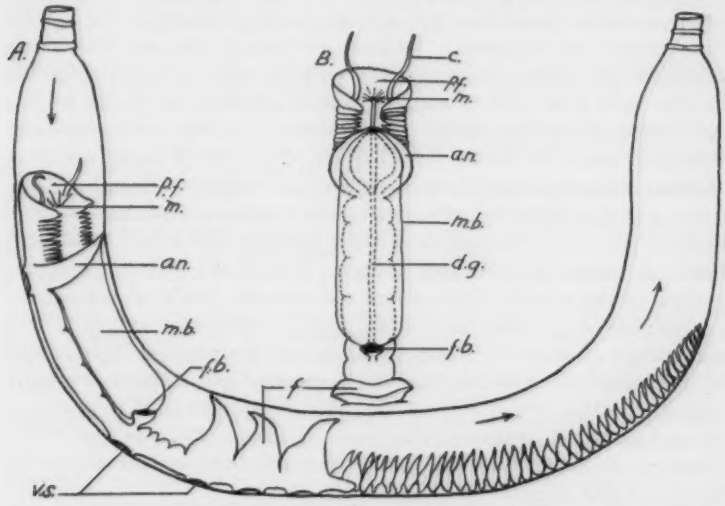

FIG. 1. *A*, *Chaetopterus variopedatus* within its tube, feeding; *B*, dorsal surface of anterior portion of worm. *a.n.*, aliform notopodium; *c.*, cirrus; *f.*, fans; *f.b.*, food ball being rolled up within the dorsal cupule; *d.g.*, dorsal ciliated groove; *m.*, mouth; *m.b.*, mucous bag; *p.f.*, peristomial funnel; *v.s.*, ventral suckers.

the walls of the burrow are completely in contact with the body of the animal and the aliform notopodia at the anterior end of the mucous bag, it is necessary for the current of water to pass into the bag, out through its sides, and thence along the body of the worm, and ultimately to issue from the burrow at the opposite end. While the current is being maintained by the fans, mucus is continuously secreted at the anterior end of the bag, and, at the same rate, the posterior end is rolled into a ball within the dorsal cupule by the cilia of its inner surface. Since all water entering the burrow while a mucous bag is

present passes through the walls of the bag, the mucus removes from the current all solid particles, whatever their size. It is these particles which lodge on the inner surface of the mucous bag that constitute the food of *Chaetopterus*. It consists mainly of detritus (organic debris and bacteria) stirred up from the surface of the ocean or estuarine bottom by wave action, currents, other animals, etc.

Because the entrances to the tube of *Chaetopterus* are considerably constricted, no very large particles find their way in with the feeding current. Such that do are usually detected by the peristomial cilia of the worm and are passed out at the sides of the worm anterior to the aliform notopodia, which are lifted to allow the material to pass, and so do not find lodgment in the mucous bag. Since the mucus of the bag is being secreted continuously, and at the same time the posterior end is being rolled into a ball in the dorsal cupule, it is evident that the entire bag is constantly being renewed, and that the posterior portion is much more heavily laden with food than is the anterior.

When the ball of mucus and food in the dorsal cupule reaches a certain size, the anterior end of the mucous bag is cut off from the notopodia, and the dorsal cupule continues to rotate the ball until the remainder of the bag is completely (or, occasionally, only partly) rolled up. The dorsal cupule is then turned anteriorly and stretched forward somewhat to expel the ball of food onto the posterior end of the dorsal groove. At the same time the action of the cilia of the groove is reversed, and the bolus of mucus with its entrapped food is carried forward along it to the mouth, where the bolus is enveloped by the lips and swallowed.

The size of the bolus of food depends upon the size of the dorsal cupule, and, therefore, upon the size of the animal. For a *Chaetopterus* about 6 inches long the food ball averages about 3 mm. in diameter. When *Chaetopterus* is feeding there is some variation in the length of its body, particularly in that portion between the head and the dorsal cupule, and, therefore, the length of the mucous bag will vary in the same animal at different times.

The following figures are given for a worm 142 mm. in length, measured during a time when the animal was feeding. Fifteen millimeters posterior to its point of origin, the width of the mucous bag was 6 mm., and the dorso-ventral diameter at the same point was 7 mm. The length of the mucous bag was 37 mm. The rate of secretion of this bag was approximately 1 mm. per second. While the worm was feeding the number of beats for any one of the three fans was 64 per minute, and this rate was the same for this particular

worm as observed on successive days over a period of several weeks. Although the rate of beating of the fans is quite uniform for any one worm, it varies with individuals, for another worm maintained a rate of 52 beats per minute. From the beginning of the spinning of the mucous bag to the ingestion of the bolus of food required, on the average, 17 minutes, and varied only plus or minus 1 minute from this average.

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THE ACTION OF EYE-STALK EXTRACTS ON RETINAL PIGMENT MIGRATION IN THE CRAYFISH, CAMBARUS BARTONI

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I

Pigment cells of the retina of the vertebrate eye and pigment cells of the compound eye of arthropods have long been known to lack motor innervation. Hence there has been much speculation regarding the nature of the mechanisms controlling the movements of these cells or the pigment within them. As recently as 1932 when Parker reviewed the literature on retinal pigments there was no direct evidence as to the nature of the control, but considerable indirect evidence suggested that hormonal agents were responsible for initiating and maintaining retinal pigment migration. The first successful attempt to demonstrate the existence of a hormone acting on the retinal pigments of arthropods was made by Kleinholz (1934; 1936). He found that the injection of an active principle from eye-stalks of *Palaemonetes* into dark-adapted individuals of the same species caused the movement of the distal and reflecting pigments to positions characteristic of the light.

Studies of the persistence, under constant external conditions, of 24-hour cycles of pigment migration in the compound eye had led to one of the earlier suggestions that there was a hormonal control of retinal pigment (Welsh, 1930; see also Welsh, 1938, for review of the literature pertaining to diurnal rhythms). The extension of these studies to the eye of *Cambarus* made necessary an investigation of hormone factors in the control of retinal pigment migration in this crustacean. Certain of the results obtained will be presented in this paper.

II

The majority of observations were made on eyes of *Cambarus bartoni* but eye-stalks of *C. clarkii* and *C. limosus* were sometimes used as sources of the pigment-activating substance.

The approximate positions of the three sets of pigment (distal, proximal and reflecting) were determined by briefly illuminating the

eye of an animal, in the dark, by a bright beam of light, and observing the amount of light reflected from the eye. This method has been employed by Day (1911). Exact determinations of pigment positions were first made by sectioning the eyes but this is a time-consuming procedure and a rapid method was developed as follows. Animals were killed by dipping in water at 80° C. for 10–15 seconds. The eyes were then removed and split in halves. When these halves were examined under a binocular, using bright reflected light, it was possible to measure the positions of the pigments quite as accurately as in sections.

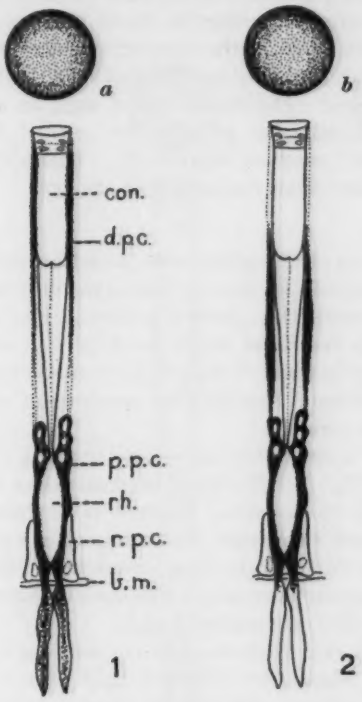
The active substance from the eye-stalk, which may be identical with the chromatophorotropic hormone (Abramowitz and Abramowitz, 1938), was prepared by grinding 20 eyes and eye-stalks of medium-sized crayfishes in 1 cc. of cold-blooded Ringer, then heating to 100° C. and filtering. The injection of an appropriate volume of the filtrate made it possible to administer the active material from a fraction of an eye-stalk or from one or more eye-stalks as a given dose. Appropriate control injections of Ringer's fluid and of extracts of ventral nerve cord were made and always with negative results.

III

The first experiments to be reported were done to test the effect of the eye-stalk extract on light-adapted eyes. Several *C. bartoni* were allowed to adapt for several hours in bright diffuse sunlight. At 10:30 A.M. 0.05 cc. of eye-stalk extract (containing the active material from one eye-stalk) was injected in the ventral abdominal musculature of each of half the individuals. At 2:00 P.M. the entire lot was killed with hot water and the eyes removed, split and examined.

In Fig. 1 may be seen the distribution of the pigments of a light-adapted eye. The distal pigment forms a sheath around the cone and the process leading from the cone to the rhabdome, but a portion of each distal pigment cell next to the retinular or proximal pigment cells is not filled with pigment. Most of the proximal pigment surrounds the rhabdome, but some remains below the basement membrane. The reflecting pigment in crayfish eyes does not migrate as it does in some crustaceans (Welsh, 1932). The appearance of an eye of a light-adapted animal, when viewed by reflected light, is shown in Fig. 1*a*. The positions of the black screening pigments are such that light cannot reach the reflecting pigment layer nor can light rays, except those which are parallel to the main axis of an ommatidium, reach the rhabdome or light-sensitive element of the eye. Such an eye is called an apposition eye since a given rhabdome receives light

only from its adjacent lens system and is not acted on by light entering at an angle through neighboring ommatidia.



EXPLANATION OF FIGURES

con. = cone
b.m. = basement membrane
d.p.c. = distal pigment cell
p.p.c. = proximal pigment cell
rh. = rhabdome
r.p.c. = reflecting pigment cell

FIG. 1. An ommatidium of a typical light-adapted eye showing the positions of the eye pigments. This and the following figures of ommatidia show the situation as seen in thin sections of the eye. In the intact light-adapted eye each cone, cone process and rhabdome is almost completely surrounded by a cylinder of pigment.

FIG. 1*a*. Showing the appearance of an intact eye with the pigment distribution seen in Fig. 1 when viewed, in the dark, by bright reflected light.

FIG. 2. An ommatidium showing the effect on the pigments of injection of eyestalk extract into a light-adapted animal.

FIG. 2*b*. The intact eye has essentially the same appearance as does the normal light-adapted eye.

An ommatidium from a typical eye of a light-adapted animal, injected with eye-stalk extract and left in the light, is represented by Fig. 2. The distal pigment is in a more extreme proximal position and all of the proximal pigment is above the basement membrane. It is as though the effect of the injected material were added to the effect of light, which probably acts by causing the release of the active material or hormone. The intact eye of such an animal has essentially the same appearance as does the normal light-adapted eye (Fig. 2*b*), although it may not be as black. This is due to the distance of the distal pigment from the surface of the eye.

IV

When specimens of *C. bartoni* were placed in the dark, the typical dark-adapted condition in the eye was seen after two hours or less. It is known, however, that there is a diurnal migration of proximal retinal pigment in crayfishes which are kept in continuous darkness (Bennitt, 1932), so in order to assure uniform conditions in all experiments on dark-adapted animals the majority of observations were made in the early evening.

The positions normally occupied by pigments in a dark-adapted eye are shown in Fig. 3. The distal pigment forms a collar surrounding the cone and the proximal pigment is all below the basement membrane. In such a condition the rhabdome of a given ommatidium may receive light from neighboring ommatidia. Such an eye is referred to as a superposition eye and is commonly found in those insects and crustaceans which are active at night.

The intact eye of a dark-adapted crayfish has a brilliant orange-red center when viewed by reflected light, due to the mirror-like property of the exposed reflecting or tapetal layer (Fig. 3*c*). The color is due to the visual red of the rhabdomes.

When dark-adapted *C. bartoni* were injected with eye-stalk extracts, and left in the dark, varying effects on the pigment were seen depending on the amount injected, and the interval between the time of injection and the time of observation. Animals which were dark-adapted for several hours and injected in the early evening with an amount of material equivalent to that obtained from one-fourth to one eye-stalk showed, after three hours, a migration of the distal pigment to or toward the light position. The proximal pigment was not affected (Fig. 4). When such eyes are viewed by reflected light they appear gray rather than black and very little light is reflected from the tapetal layer (Fig. 4*d*).

The injection of 0.1 cc. of the extract (= the extractible material from two eye-stalks) had a distinct effect on the proximal as well as

the distal pigment. After three hours both pigments were found to occupy positions more or less typical of light adaptation (Fig. 5). The intact eye when viewed by reflected light had the same appearance as the normal light-adapted eye (cf. Fig. 5*e* with 1*a*).

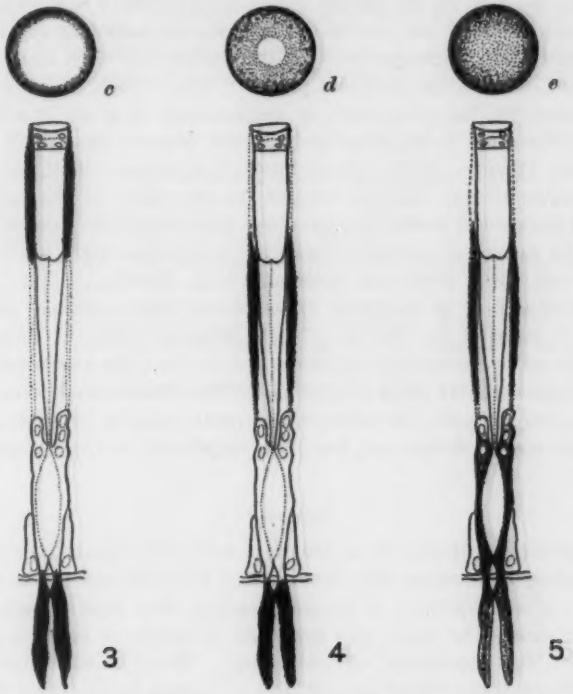


FIG. 3. Ommatidium of a typical dark-adapted eye showing the positions of the pigments.

FIG. 3*c*. The intact eye of a dark-adapted animal has a bright orange-red center when viewed by reflected light.

FIG. 4. The injection of the active material from one eye-stalk into a dark-adapted animal causes the migration of the distal pigment to the light position.

FIG. 4*d*. The intact eye of such an animal may have a small reflecting central area.

FIG. 5. The injection of the active material from two eye-stalks into a dark-adapted animal causes the migration of both distal and proximal pigments to their light positions.

FIG. 5*e*. The intact eye of such an animal has the same appearance as that of a light-adapted animal.

V

It has been demonstrated that it is possible, by means of a simple extraction process, to obtain from two eye-stalks of a crayfish an amount of retinal pigment activator, or hormone, equivalent to that normally released by the animal during the process of light adaptation. From one eye-stalk the amount of hormone is sufficient only to activate the distal pigment cells; thus indicating that they have a lower threshold than do the proximal pigment cells. Such threshold differences between the three sets of pigments in a given species may account for such a situation as was first seen in the eye of *Macrobrachium* (Welsh, 1930), where under continuous illumination the distal pigment cells migrate toward the periphery of the eye at the time of sunset and return to a proximal position at the time of sunrise, while the proximal pigment remains in a constant light position (see also Welsh, 1935, 1936; and Kleinholz, 1937, 1938).

The injection of eye-stalk extracts into dark-adapted crayfishes makes it possible to obtain a "light-adapted" eye, as regards the positions of the screening pigments, while the "dark-adapted" level of the light-sensitive substance of the retina remains unaffected. This enables one to study the effect of pigment position on visual acuity and response to flicker and has been employed by Crozier and Wolf (1939).

Summary

A substance similar to, or identical with, the eye-stalk or chromatophorotropic hormone may be obtained from the eye-stalks of crayfishes. When injected, in proper amount, into light-adapted crayfishes it causes the distal and proximal pigments to migrate to more extreme "light positions" than normal. When injected into dark-adapted crayfishes which are allowed to remain in the dark it causes the migration of one or both sets of screening pigment to their "light positions." The distal pigment has a lower threshold than the proximal pigment, as it is affected by lower concentrations of the active substance. It is suggested that such threshold differences may account, in part, for the unusual pigment responses which have been observed in compound eyes in studies of 24-hour cycles in pigment migration.

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THE FLICKER-RESPONSE CONTOUR FOR THE CRAYFISH

II. Retinal Pigment and the Theory of the Asymmetry of the Curve

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I

The flicker-response contour ($F - \log I$) for the crayfish *Cambarus bartoni* resembles that for other arthropods having markedly convex eyes (see Crozier and Wolf, in press). Only its very uppermost part can be fitted by a probability integral. Over its lower part the slope increases too rapidly, so that the whole curve is quite asymmetrical. This departure from the rule observed in the responses of vertebrates (see Crozier and Wolf, 1937*a* and *b*, 1938*a*) has been accounted for (Crozier and Wolf, 1937*c*, 1938*b*) by the shape of the optic surface in the majority of arthropods. With increasing flash-intensities the retinal area effectively involved is increased, which results in a higher F; this is due to the greater chance of exciting ommatidia toward the circumference of the curved eye. Confirmation of this view, consistent with the consequences of changing the light-time fraction in the flash-cycle (Crozier and Wolf, 1937*c*, 1938*b*), is given by the fact that an arthropod with sufficiently flat optic surfaces, the isopod *Asellus* (Crozier and Wolf, 1939), gives a flicker-response contour which is a perfectly symmetrical probability integral. The asymmetry of the curve with *Anax* is appropriately reduced by blocking out all but a central area of the eye (Crozier and Wolf, 1937*c*, 1938*b*), and in a form with still more markedly curved optic surfaces (*Cambarus*) (see Crozier and Wolf, in press) the asymmetry is much more extreme.

In our experiments with *Anax* (Crozier and Wolf, 1937*c*, 1938*b*) the limitation of the increase of effective retinal area with increase of illumination by painting portions of the eyes was recognized to be imperfect. A certain amount of leakage of light near the margins of a cap of enamel, and under its edge, cannot be prevented. A neater method of accomplishing the purpose is to use the migrations of retinal pigment cells. The flicker-response contours we have discussed were determined with animals previously dark-adapted. For such a crustacean as *Cambarus* this means that the proximal retinal pigment is below the level of the receptive retinulæ, the distal pigment cells well

out toward the surface of the eye around the crystalline cones. The retinulæ are completely unshielded from laterally spreading light, and the condition is that for the "superposition" type of eye (Exner, 1891). In the eye well light-adapted the forward migration of the proximal pigment shields the retinulæ, while the inward movement of the distal pigment forms around each ommatidium an opaque tube of pigment along the length of the crystalline lens and down to the proximate pigment (Bernhards, 1916; Day, 1911; Parker, 1932). The effective isolation of each recipient unit from light other than that proceeding down the axis of the ommatidium then produces the condition for the "apposition eye" (Exner, 1891).

For our purposes, however, no use could very well be made of the control of retinal pigment migration by light. The process of light adaptation involves not only movements of the retinal pigment cells, but also, it must be presumed, the intrinsic photic adaption of the visual response system itself. At the same time, if some other procedure could be found to cause the retinal melanophores to assume the "light-adapted" condition, it should serve admirably for a test of certain properties of the *Cambarus* flicker-contour. It should also give some direct behavioral evidence as to the functional rôle of the retinal pigment and its movements, as well as providing material for a logical approach to the method of estimating the time-course of visual light-and-dark-adaptation in such animals.

It was pointed out to us by Dr. J. H. Welsh that extracts containing the "eyestalk hormone" from the optic peduncle produce an effect on the melanophores and also on the movement of retinal pigment (Kleinholz, 1934, 1936, 1938; Welsh, 1939) in dark-adapted eyes of *Cambarus*, so that injection of sufficient extract into a dark-adapted animal leads to the migration of retinal pigment into positions characteristic of the normal light-adapted state. This we have verified in *C. bartoni*.

II

The observational procedure was identical with that employed in measuring the flicker-response contour for dark-adapted *Cambarus* (Crozier and Wolf, in press): temperature 21.5°, 50 per cent light-time in the flash cycle. To keep the handling of the animals uniform with respect to time after injection and the like, a lot of 5 rather than of 10 was used. The eyestalks from 10 *Cambarus bartoni* were extracted in Ringer solution. Into each crayfish prepared for observation there was injected into the abdomen 0.08 ml. of extract, the equivalent of 2 eyestalks. After 75 to 90 minutes in the dark the crayfish are

bluish in body color and by means of a beam of light directed into the eye the retinal pigment is seen to be in the position characteristic of light adaptation. Sectioned eyestalks fixed in hot water at this stage show the condition clearly under the ultrapak microscope. In the normal dark-adapted eye the proximal pigment is retracted below the basement membrane, while the distal pigment is out between the crystal cones. There is no detectable pigment between the ommatidial units. After about 90 minutes in darkness subsequent to injection of eye-stalk extract, the proximal pigment surrounds the

TABLE I

Data for the flicker-response contour of the crayfish *Cambarus bartoni*, with eye-pigment in the "light adapted" state as result of injection of eye-stalk hormone. $N = 5$ individuals, $n = 3$ observations on each; the same individuals used throughout; $t° = 21.5°$ C.; $t_L = t_D$. See Fig. 1. I in millilamberts, F in flashes per second. P.E.$_1$ = P.E. of the dispersions.

F	F_m	$P.E._{1F_1}$	$\log I_m$	$\log P.E._{1I_1}$
2			$\bar{3}.3404$	$\bar{5}.9958$
5			$\bar{2}.0777$	$\bar{4}.3334$
8			$\bar{2}.2584$	$\bar{4}.4806$
12			$\bar{2}.5249$	$\bar{4}.8155$
16			$\bar{2}.6674$	$\bar{4}.9737$
20			$\bar{2}.7771$	$\bar{3}.0398$
25			$\bar{2}.9254$	$\bar{4}.8291$
30			$\bar{1}.0730$	$\bar{3}.2355$
35			$\bar{1}.2865$	$\bar{3}.6169$
40			$\bar{1}.5937$	$\bar{3}.8785$
	41.79	0.289	0.00	
42			0.0233	$\bar{2}.3664$
43			1.0077	$\bar{1}.2823$
	43.10	0.357	0.50	
	43.23	0.361	1.00	
	43.66	0.282	1.25	
44			1.5231	0.1464
	44.05	0.130	1.50	

retinulæ, while the distal pigment now envelopes each ommatidial unit down to its base. The condition is one of quite complete shielding of each ommatidium by a dense layer of black pigment, more extreme than is the case in ordinary light adaptation.

III

The determinations of mean critical flash-intensity and mean critical flash-frequency for response (Crozier and Wolf, in press) to visual flicker are given in Table I. Comparison with the results for normally

dark-adapted *Cambarus bartoni* (Crozier and Wolf, in press) shows that there is a pronounced (reversible) effect of the injection of eyestalk hormone upon the properties of the flicker-response contour. This cannot reasonably be traced to an effect of the eye-stalk extract upon the intrinsic processes of photic excitability, for several reasons. In

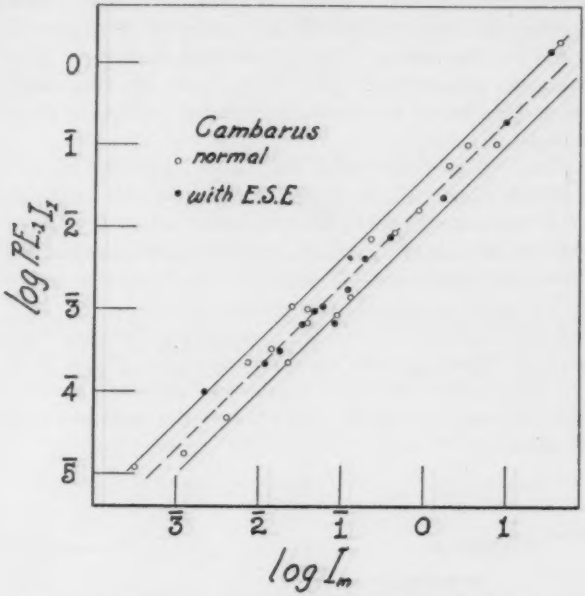

FIG. 1. The variation of I_1 for normal *Cambarus bartoni* and after injection with eye-stalk extract (*E.S.E.*); Table I; see text.

the first place injection of *ca.* 0.06 ml. of the eyestalk extract into *Anax* (dragon fly) nymphs produces no detectable effect either on pigment migration or on the flicker-response curve, as the following observations showed (tests on 5 individuals):

F	Normal log I_m	Normal + eye-stalk extract log I_m
20	2.473	2.478
30	2.749	2.745
	2.741	2.750

Any effect of this sort would thus have to be specific. In the second place, the results of adapting *Cambarus* are rapidly apparent even when the retinal pigment is already fully advanced into the "light" position, as subsequently shown (§ IV). Finally the various modifica-

tions of the flicker-response contour are those to be expected as the result of the optical shielding of the ommatidia, so that no specific effect on excitability need be invoked.

For any given level of flash-intensity the variation of I_1 among the individuals used is statistically of the same magnitude as for the normal group previously examined (Crozier and Wolf, in press). The 5 individuals giving the data of Table I were in the lot of 10 providing the normal curve for this species (Crozier and Wolf, in press). The scatter of the variation indices (P.E.$_{I_1}$) is even a little less than might have been expected in view of the smaller number of readings in the eyestalk injection series (Fig. 1).

The effects to be expected if the "dark" position of the retinal pigment shields ommatidia from all but light parallel to the retinular axis, and if this is to prevent the recruitment of optic impulses from a larger retinal surface as flash intensity is increased, are the following: (1) the total achievable sensory effect ($= F_{max.}$) must be reduced; (2) at given I, F must be less; (3) the asymmetry of the $F - \log I$ curve must be markedly reduced; and (4) it would not be surprising to find the slope of the "fundamental" curve increased (i.e., $\sigma'_{\log I}$, for the ideal frequency distribution of log I thresholds, *reduced*), owing to the mechanical exclusion of a large proportion of the otherwise marginally excitable units.

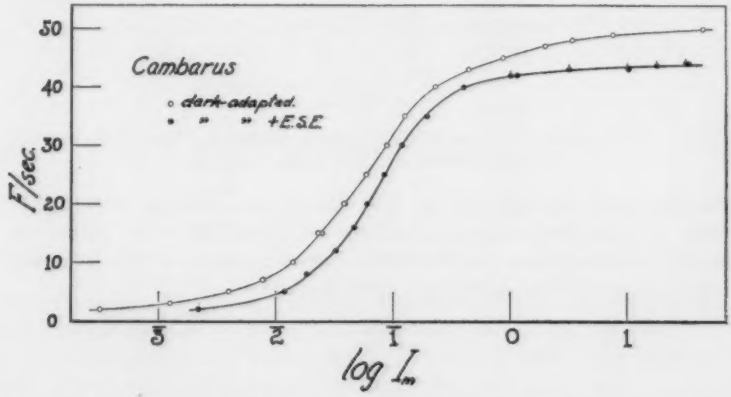

FIG. 2. *F*—log *I* curves for dark-adapted *Cambarus* and under the same conditions for individuals injected with eye-stalk extract (*E.S.E.*); Table I.

Figure 2 shows that the $F - \log I$ curve with *Cambarus* dark-adapted but under the influence of eye-stalk extract is moved toward higher intensities and exhibits a lower maximum. These are the

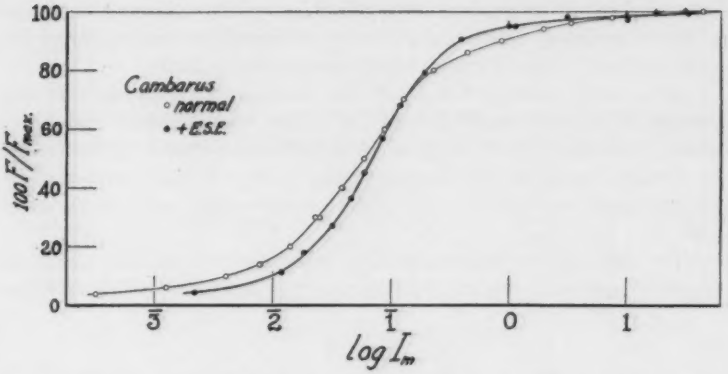

FIG. 3. The curves of Fig. 2 brought to the same $F_{max.}$ (= 100 per cent), to show change of shape.

results of a decrease in the total number of excitable elements (Crozier and Wolf, 1937*c*, 1938*b*), as expected.

The asymmetry of the curve is also decreased (Fig. 3). The

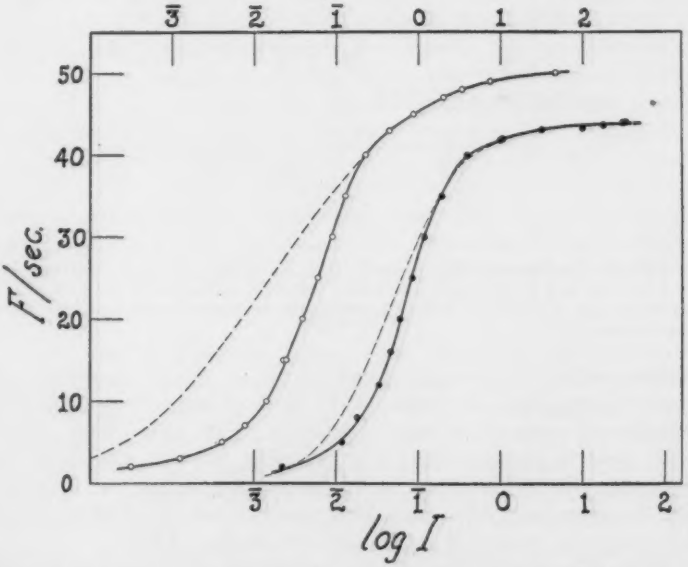

FIG. 4. The curves of Fig. 2 with probability integrals adjusted to the upper portions (*cf.* Crozier and Wolf, 1937*c*, 1938*b*, 1939, and paper in press), to show that the flicker-response contour after injection of eye-stalk extract departs less than the normal; see text.

sheathing of the ommatidia by pigment materially reduces the chance of photic action on additional elements as intensity increases, hence the slope of the $F - \log I$ curve cannot increase so rapidly.

It is to be presumed that in the absence of comparatively free passage of light through the eye (as in the dark-adapted state), the actual intensity at each receptor locus will be decreased. This cannot be a major factor in the changes shown in Fig. 2, else increase of intensity would find the $F - \log I$ curve continuously rising at its upper end.

The diffusion of light within the substance of the eye cannot be ignored, however. Figure 4 shows that the asymmetry of the flicker-

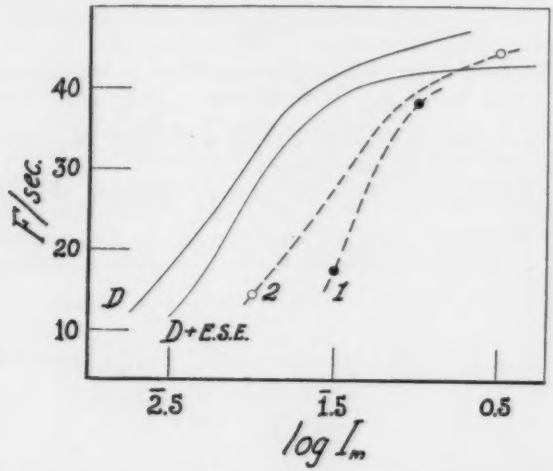

FIG. 5. Portions of the curves in Fig. 2 for dark-adapted *Cambarus* with (*D* + *E.S.E.*) and without (*D*) injection of eyestalk extract, and the first (1) and second (2) sets of readings (Table II) during the progress of dark adaptation after light adaptation.

response contour has been decreased (*cf.* Fig. 3), but not abolished. In view of the proximal movement of the distal retinal pigment under the influence of eye-stalk extract (Kleinholz, 1934, 1936, 1938; Welsh, 1939), this is not surprising. It probably explains the slight but detectable rise of the curve at the highest intensities used (Table I; Fig. 2), particularly when F_m is determined at constant flash-intensity; this cannot be accounted for by light adaptation (§ IV).

With allowance for this effect, a reasonable adjustment of an ideal probability integral can be made to the upper part of the curve (Fig. 4).

Comparison with the normal, in the same figure, shows that $\sigma'_{\log I}$ is, as expected, much reduced.

IV

Light adaptation of *Cambarus* reduces the $F - \log I$ curve (Table II, Fig. 5); with even brief residence in darkness the curve rises toward

TABLE II

Critical flash-frequencies at two flash-intensities for *Cambarus:* (1) very shortly after light adaptation to bright daylight; (2) after *ca.* 10 minutes in darkness; 3 observations on each of the same 4 individuals at all points; 21°.5, $t_L = t_D$. See text, and Fig. 4.

	$\log I$	F_m	P.E.$_{F_1}$
(1)	$\bar{1}.50$	17.5	0.491
	0.00	38.3	0.371
(2)	$\bar{1}.00$	14.5	1.52
	0.50	44.5	4.81

the position typical for dark adaptation (Fig. 5). Obviously, for a quantitative investigation of the kinetics of photic excitation, the effect of the migration of retinal pigment as governed by light and darkness must be ruled out. The present data supply the first evidence of a functional rôle of the position of the retinal pigment in matters of visual response. The result of light adaptation, as with certain other forms, is to reduce F at fixed I, but to follow by this means the recovery of excitability during subsequent darkness is made difficult by the fact that the retinal pigment also changes position. Either the pigment must be held in a fixed position throughout, by suitable repeated injection of eye-stalk extract, or else a procedure found for extrapolation to a constant condition of the pigment.[1] The latter could perhaps be achieved by determining the relation between the position of the $F - \log I$ curve and various known positions of the pigment; in any event the whole course of the function must be known.

SUMMARY

Injection of *Cambarus bartoni* with extract of eyestalks of this species forces migration of retinal pigments of individuals kept in darkness into positions characteristic of the light-adapted eye. In this condition the receptor elements of each ommatidium are effectively shielded from light passing through their neighbors. The flicker-response contour then differs in four particulars from that found when the retinal pigment is in the "dark" position, for which effective screen-

[1] For a somewhat analogous case of changing sensitivity during the interval of observation, a technic of this kind was used with *Agriolimax* (Crozier, W. J., and Wolf, E., 1928–29, *Jour. Gen. Physiol.*, 12: 83).

ing of the ommatidia is not present: $F_{max.}$ is lowered; the whole curve is moved to higher intensities; the spread of the log I thresholds for the cumulative population of sensory effects is lessened; and the asymmetry of the $F - \log I$ curve is markedly reduced. It is pointed out that these results are to be expected if the asymmetry of the curve in normal dark-adaptation is due to the relation between flash-intensity and the curvature of the optic surface and divergence of the ommatidial axes.

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THE SIGNIFICANCE OF GERMARIA IN DIFFERENTIATION OF OVARIOLES IN FEMALE APHIDS

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Introduction

Recognition of the fact that winged parthenogenetic female aphids produce both parthenogenetic and gamic female offspring differing in part in the structure of the ovarioles, invites an understanding of the mechanism that controls the development of the ovarioles. Such an undertaking may aid in determining how genes, presumably identical, can produce two types of individuals.

In studies on the development of aphids (Lawson, 1939), it was noted that the germaria are the first embryonic structures to mark a distinction between gamic and parthenogenetic females. Other differentiating characters do not appear until after birth. As determination of all differentiating characters occurs before birth (Shull, 1930*a*), it is possible that the germaria are instrumental in determining the adult nature of the individual at least in so far as the ovarioles are concerned.

In female aphids the essential reproductive organs consist of a pair of ovaries in which the eggs are developed, and an oviduct leading from each ovary to an external opening. Each ovary is made up of a number of loosely parallel ovarian tubes (ovarioles) which open into the oviduct. Three different regions are recognized in an ovariole (Fig. 1),—the terminal filament, the germarium, and the vitellarium. The terminal filament is a thread-like structure at the end of the ovariole farthest from the oviduct, which attaches the ovariole to the body wall. Next behind the terminal filament is the germarium which contains the germ cells from which the eggs develop, and nurse cells whose function is to furnish nutriment to the developing eggs. The vitellarium is a tubular structure which extends from the germarium to the oviduct and contains developing eggs in a gamic female and both eggs and embryos in a parthenogenetic female. A nutritive thread or yolk stream extends from the nurse cells to the youngest growing oöcyte in the vitellarium.

Differences between Gamic and Parthenogenetic Females

Gamic females are described by Shull (1930*b*) as follows: "Gamic females of this species of aphid have a wax yellow body color, dark

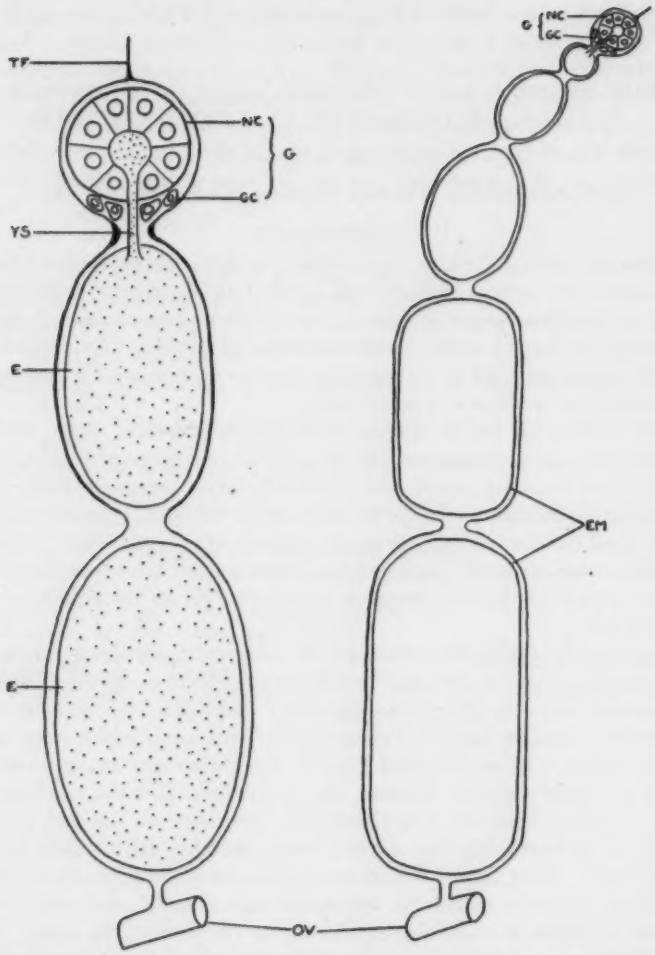

FIG. 1. Diagrams of aphid ovarioles, gamic at left, parthenogenetic at right. *E*, egg; *Em*, embryo; *G*, germarium; *GC*, germ cell; *NC*, nurse cell; *OV*, oviduct; *TF*, terminal filament; *YS*, yolk stream.

brown antennae, and greatly swollen hind tibiae of dark brown color, covered with hundreds of sensoria. Their reproductive systems consist of a vagina on which are borne a pair of colleterial glands and a seminal receptacle, a pair of short oviducts formed as branches of the vagina, and a variable number (usually ten) of ovarioles branching

from the oviducts. Each ovariole, in a mature female, contains usually one mature or nearly mature egg, distinctly opaque and of very regular ovoid form; beyond this often an oöcyte, in early growth stage, hence long and slender and not very opaque; and lastly a large, spherical germarium forming a conspicuous knob at the end of the ovariole. In old gamic females, especially those that have not been laying eggs, the second reproductive cell from the base of the ovariole may be large and opaque and regular in form, and is then presumably mature like the one posterior to it. Almost never, however, in typical gamic females (that is, those produced at low temperature by winged females whose other daughters are practically all gamic), are there more than two oöcytes in any stage in one ovariole. An ovariole of a gamic female may therefore be regarded as regularly consisting of a tube containing one or two eggs or oöcytes, and a large round germarium."

In stained sections of adult gamic females the ovarioles (Fig. 1) are prominent in the abdominal cavity. The germaria usually lie anterior to the large yolk-laden eggs. Each germarium is surrounded by the closed end of an ovariole tube composed of a single layer of thin, squamous epithelial cells. Posterior to the germarium the tube constricts, forming a short neck in which the lumen is quite narrow. The narrowing of the lumen is due in part to contraction of the tube, but also to an increase in the height of the cells which change from a squamous to a columnar type in the neck region. A constriction of the tube likewise occurs between the eggs contained in the vitellarium, but this is due to contraction only as there is no change in cell shape. Around the young growing oöcytes the ovariole wall is constructed of a single layer of cuboidal cells. In the posterior part of the ovariole the cells are rectangular with the greatest width parallel to the surface of the egg.

The germarium is composed of two types of cells, a round ball of large nurse cells and a small group of germ cells. Each nurse cell is roughly pyramidal in shape (triangular in section) with the base at the periphery of the germarium and the apex in the center. The nurse cells fit closely together and form a ball, in the center of which is a substance secreted by the nurse cells. This substance flows from the central area through the neck of the ovariole into the growing oöcyte within the vitellarium. The exact nature of this substance is unknown, however, as it flows directly into the growing oöcyte; it might be yolk. No better term is available, hence the term "yolk" is used to facilitate discussion, and the term "yolk stream" is used to indicate the distinct cord, or string of substance which passes from the germarium into the growing oöcyte.

Germ cells, smaller and fewer in number than the nurse cells, lie between the ball of nurse cells and the neck of the ovariole. Posterior to the neck the vitellarium contains ovoid oöcytes in various stages of growth. Because only young adults were used for this study, it is likely that no mature eggs were examined. Each oöcyte consists of a central mass of yolk surrounded by a thin peripheral layer of cytoplasm. Outside of this cytoplasm is a single egg membrane. In the smaller younger oöcytes the nucleus occupies the center of the cell, but in older ones it lies at the periphery halfway between the two ends of the egg. The nucleus is large and clear and contains but a few small bodies which stain heavily.

Parthenogenetic females are described by Shull (1930*b*) as follows: "The parthenogenetic females have bright green body color, antennae quite pale except in the distal segment, and very slender, pale hind tibiae bearing no sensoria. The reproductive system consists of a vagina, without collegerial glands or seminal receptacle, two short oviducts branching from the vagina, and a variable number (apparently up to ten) of ovarioles branching from the oviducts. Each ovariole is a very delicate tube containing, in healthy individuals, usually six to nine embryos or eggs or oöcytes, and bearing at the end a very small germarium which is usually not much larger, and is often smaller, than the oöcyte or egg next behind it. All of these reproductive elements except one (the one next to the germarium) are as a rule embryos in some stage of development. They are all translucent or transparent, unless dead, and if dead they have a clouded appearance not at all like the opaque gamic eggs. Only the smaller embryos are ellipsoidal; the medium and larger ones always possess angles which correspond to the form of the young aphids. The six to nine embryos or eggs in one ovariole, in a typical healthy female, are of regularly decreasing size from oviduct to germarium, so that they resemble a tapering string of beads."

In stained sections of parthenogenetic female aphids the entire abdominal cavity is crowded with embryos of varying sizes and stages of development. In general, the larger more developed embryos lie in the posterior region while the smaller less developed embryos lie anterior to them. The germaria (Fig. 1), small and difficult to locate, usually lie in the anterior abdominal region squeezed among young embryos or between the embryos and the lateral body wall. The wall of the ovariole tube around the germarium is a single squamous epithelium which constricts, forming a neck just posterior to the germarium. The cells become somewhat cuboidal in this region. Extending posteriad from the neck the ovariole wall encloses embryos,

and is composed of a thin single layer of squamous cells. The tube is always constricted between the embryos within any one ovariole. The parthenogenetic germarium consists of two types of cells, a round ball of nurse cells and a small group of germ cells. Each nurse cell is roughly pyramidal in shape with the base at the periphery of the germarium and the apex in the center. The nurse cells fit closely together and form a ball. This ball of nurse cells contains yolk, and a yolk stream extends from the nurse cells through the ovariole neck into the youngest growing oöcyte. The germ cells lie in the germarium between the ball of nurse cells and the neck of the ovariole. Young oöcytes are found immediately posterior to the germarium. Progressing caudad, the next in line is usually an egg in cleavage followed in turn by a young developing embryo. Thereafter each succeeding embryo is larger and more fully developed.

Major Differences between Adult Gamic and Parthenogenetic Ovarioles

The primary difference between gamic female and parthenogenetic female ovarioles is in the development of the germ cells. In gamic female ovarioles development of germ cells consists of growth through accumulation of yolk and possibly meiosis, though no divisions have been observed in this species of aphid. In the parthenogenetic female ovarioles the germ cells are stimulated to develop parthenogenetically.

The size difference in the germaria presumably is secondary to and correlated with the germ cell difference. Gamic female ovarioles contain eggs that undergo embryonic development outside of the mother's body, are dependent on a large yolk supply and consequently accumulate this yolk supply during growth in the ovariole. The large size of the gamic female germarium (about three times larger than a parthenogenetic female germarium in fixed material) is evidently correlated with the necessity of producing much yolk. Eggs produced by parthenogenetic females develop within the body of the mother, and it is probable that nourishment for this growth and development is supplied directly by the mother by body fluid. Thus a large quantity of yolk is unnecessary for parthenogenetic eggs, and the small size of parthenogenetic female germaria may be correlated with this decreased secretory activity.

Ovarioles of Gamic Female and Parthenogenetic Female Embryos

The ovarioles of gamic female and parthenogenetic female embryos were studied and compared in those embryos of both types which

showed the greatest degree of development. These embryos were well developed, occupied the posterior abdominal region of the mother and, presumably, would have been born very shortly had the mother been allowed to live.

Each ovariole in gamic embryos of this late stage of development consists of a terminal filament, a germarium and a vitellarium (Fig. 2). Each germarium contains nurse cells and germ cells. The nurse cells are roughly pyramidal in shape and form a ball at the end of the

FIG. 2. Photomicrograph of an embryonic ovariole of a gamic female aphid. *G*, germarium; *GC*, germ cell; *NC*, nurse cell; *V*, vitellarium.

ovariole. This is similar to the adult condition although the cells seem to be more loosely packed and the entire germarium is more elongated than in the adult. The center of the ball of nurse cells contains yolk, but there is no yolk stream. Germ cells occupy the region of the germarium posterior to the nurse cells. The ovariole tube surrounds the germarium as a simple squamous epithelium and continues posteriad as a small tubular vitellarium with a narrow lumen. The vitellarium contains no eggs or oöcytes.

In parthenogenetic female embryos of the same degree of develop-

ment the ovariole consists of a terminal filament, a germarium, a vitellarium, and parthenogenetically developing germ cells (Fig. 3). Each germarium consists of nurse cells and germ cells. The nurse cells form a ball of cells at the tip of the ovariole tube within which is found a small amount of yolk. A yolk stream extends from the center of the ball of nurse cells through the neck of the ovariole into the youngest growing oöcyte. The germ cells lie just behind the nurse cells. Each germarium is surrounded by the closed end of the ovariole tube which

FIG. 3. Photomicrograph of an embryonic ovariole of a parthenogenetic female aphid. *E*, egg undergoing cleavage; *G*, germarium; *O*, oöcyte.

consists of a thin squamous epithelium. The ovariole tube continues posteriad of the germarium as a thin-walled vitellarium which invariably contains a growing oöcyte just behind the germarium and an egg undergoing cleavage behind the oöcyte.

To insure that embryos identified as gamic females were actually gamic, parthenogenetic winged females were fixed when they were producing gamic females only. The parthenogenetic female embryos were studied only in wingless parthenogenetic females which seldom produce gamic females. The offspring of both types of parents produced before fixation were reared and examined.

Do Germaria Control Differentiation of Ovarioles?

The possibility that germaria play a significant rôle in the differentiation of ovarioles is indicated by three observations. (1) Adult ovariole differences are due primarily to the type of egg development that occurs within the ovariole and to the size of the germarium. (2) Germaria contain and produce the germ cells that develop within the ovarioles. (3) Germaria are the first reproductive structures to be differentiated in the embryo (Lawson, 1939).

Determination of an embryo into either a gamic or parthenogenetic female must occur sometime during parthenogenetic development between the growth of the oöcyte and the differentiation of the germaria. This determination may affect the embryo in two ways. (1) It may include the entire embryo in its effect so that the differentiation of the germaria would simply represent the first differential reaction of a general condition throughout the embryo. This would exclude the germaria from any significance in future development. (2) It may occur at the time that germaria are developing and affect them only. According to this assumption the embryo would be potentially capable of developing into either a gamic or a parthenogenetic female prior to determination of the germaria. After this event the aphid (more specifically the ovarioles) would become gamic or parthenogenetic depending on the nature of the germaria.

The study of normal gamic and parthenogenetic female aphids offers no choice between the two ways in which determination may occur. However, aphids intermediate between gamic and parthenogenetic are occasionally produced and an analysis of these intermediates gives us a choice.

These intermediates, described by Shull (1930*b*), show the intermediacy in several structures of which the ovarioles only are to be considered here. The intermediacy is expressed in the ovarioles in a very irregular fashion so that no two aphids with intermediate ovarioles are necessarily identical. An intermediate aphid may be a mosaic with respect to the ovarioles, in that one or more of the ovarioles are strictly gamic while the others are strictly parthenogenetic. Any one ovariole may be intermediate in that the germaria are smaller than normal gamic germaria, but larger than normal parthenogenetic germaria. The contents of the vitellarium may be intermediate in three different ways. (1) Eggs may vary from gamic in being less opaque than strictly gamic eggs. (2) Gamic eggs may occur in greater numbers than is normal for a gamic ovariole. (3) Embryos characteristic of parthenogenetic ovarioles may be abnormal. According to Shull's description, it is possible to have any combination of the above conditions in one aphid.

The first type of intermediate mentioned here in which one individual contains both gamic and parthenogenetic female ovarioles could not be produced unless the ovarioles are able to develop within the aphid independently of one another. As the germaria appear in the embryo prior to other ovariole structures, it follows that the germaria also must develop and be determined independently of one another.

The second type of intermediate in which the germaria are intermediate in size between gamic female and parthenogenetic female germaria indicates that the mechanism of determination is such that intermediate germaria are determined and differentiated within the embryo as well as germaria that are strictly gamic or parthenogenetic.

The mechanism of determination suggested by Shull (1930*a*), in which a high level of some substance within the embryo produces one type while a low level of the same substance produces the opposite type, could very easily account for intermediate germaria. These intermediate germaria could result from a condition in which determination of the germaria occurred when the level of concentration of the determining substance was intermediate between the high and low extremes.

The remaining types of intermediate aphids show the intermediacy in the ovariole contents. All of these can be explained by assuming that the germarium attached to the end of each intermediate ovariole is intermediate. As gamic female germaria produce much yolk while parthenogenetic female germaria produce little yolk, it would be expected that intermediate germaria would produce an amount of yolk intermediate between the gamic and parthenogenetic extremes.

One of the types of intermediate ovarioles described by Shull had eggs that were gamic but less opaque than strictly gamic eggs. Such eggs could be produced by a germarium that was gamic with respect to the type of eggs produced but intermediate with respect to yolk production. The decreased amount of yolk in the eggs might make them less opaque than normal gamic eggs.

Another type of intermediate ovariole had gamic eggs in greater numbers than the typical one or two of strictly gamic ovarioles. One characteristic of gamic germaria is that they produce no more than two eggs while parthenogenetic germaria produce many more than two. The above intermediate ovariole could have resulted from a germarium that was intermediate with respect to the number of eggs produced, while at the same time it was gamic with respect to the type of eggs produced.

The last type of intermediate described by Shull contained par-

thenogenetic ovarioles in which the embryos were abnormal. The details of the abnormality were not described but it is possible that the abnormality could have been due to an intermediate germarium in which parthenogenetic oöcytes or eggs were produced plus a quantity of yolk greater than is normal for a parthenogenetic germarium. This abnormal amount of yolk very likely would interfere with normal embryonic development and produce abnormal embryos.

The theory is proposed that germaria are determined independently of one another and also of the rest of the aphid embryo, that a single germarium may be caused to develop into a gamic female type, a parthenogenetic female type or a type intermediate between the gamic and parthenogenetic types. It is proposed, further, that the germarium once determined, controls the differentiation of the ovariole to which it is attached and thus controls, in part, the development of the adult aphid type.

Summary

Winged parthenogenetic female aphids produce both parthenogenetic female and gamic female aphids.

The ovarioles of adult gamic female and parthenogenetic female aphids differ primarily in the nature of the eggs developing within them and secondarily in the size and secretory activity of the germaria. Gamic female germaria are large and secrete much yolk; parthenogenetic female germaria are small and secrete little yolk.

The ovariole differences apparent in the adult aphids are also evident in the embryos. In parthenogenetic female embryos of a late stage of development the embryonic ovarioles already contain oöcytes and eggs undergoing parthenogenetic development while in the gamic female embryos of the same stage of development the germ cells have not yet entered the vitellarium. The germaria of parthenogenetic female embryos are smaller than the germaria of the gamic embryos of the same stage of development.

In both gamic and parthenogenetic female embryos the germaria are the first reproductive structures to develop.

The theory is proposed that determination of the ovariole type (either gamic female or parthenogenetic female) affects the germaria only. Each germarium, thereafter, controls the development of the ovariole to which it is attached.

Aphids intermediate between gamic female and parthenogenetic female aphids with respect to the ovarioles are described and analyzed to support the above theory.

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